RELATIONSHIP OF BE2 SURFACE ANTIGEN EXPRESSION TO T-CELL CLONALITY IN CUTANEOUS T-CELL LYMPHOMA AND SCLERODERMA

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1990

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Mittie Elizabeth Kelleher
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ABSTRACT

RELATIONSHIP OF BE2 SURFACE ANTIGEN EXPRESSION TO T-CELL CLONALITY IN CUTANEOUS T-CELL LYMPHOMA AND SCLERODERMA

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BE2 is a mouse monoclonal antibody raised against malignant CD4+ T-cells from a patient with cutaneous T-cell lymphoma (CTCL). Previous studies by Edelson et al suggested that BE2 expression serves as a marker of response to photopheresis in the treatment of CTCL, with a fall in the percentage of BE2+ lymphocytes correlating with clinical response. Subsequently, detection of BE2 on as much as 30-40% of lymphocytes from some patients with scleroderma (PSS) was one factor which prompted a trial of photopheresis for the treatment of that disease. The role of BE2 in the response of patients to photopheresis is unknown, and the confirmation of its presence as a marker of the malignant clone in CTCL is unconfirmed. We have investigated the significance of BE2 antigen expression in identifying a clonal T-cell population in patients undergoing photopheresis for treatment of CTCL and PSS. Peripheral blood lymphocytes from 5 patients with leukemic phase CTCL were sorted by flow cytometry to separate BE2+ and BE2- cells, and analyzed by Southern blot analysis of the T-cell receptor ß chain genes. Samples from all 5 patients showed monoclonal rearrangements of the T-cell receptor ß chain genes; however, there was no difference in the percentage of monoclonal cells in the BE2+ and BE2- populations. Further, DNA was analyzed from 6 patients with PSS who had at least 20-30% BE2+ peripheral

blood lymphocytes for evidence of a monoclonal T-cell popluation. All 6 patients had germline configurations of the ß chain genes. These studies confirm that BE2 antigen expression is not a marker restricted to a monoclonal population in these two groups of patients. In addition, alterations in expression of BE2, although perhaps a marker of clinical response to photopheresis in CTCL, probably do not correlate directly with cytotoxic activity against the malignant clone.

TABLE OF CONTENTS

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INTRODUCTION

Cutaneous T-cell lymphoma (CTCL), a malignant neoplasm of CD4+ lymphocytes, begins as a cutaneous disease which then progresses to a leukemic phase with systemic involvement.[1] BE2 is a monoclonal antibody which was produced by immunizing mice with malignant CD4+ lymphocytes from a patient with CTCL.[2] In a paper demonstrating the efficacy of extracorporeal photochemotherapy (photopheresis) in the treatment of CTCL, Edelson et al suggested that BE2 was useful as a laboratory marker of response to therapy with patients responding to photopheresis demonstrating marked decreases in the percentages of their lymphocytes expressing the BE2 surface antigen.[3] Although the exact mechanism of photopheresis has yet to be elucidated, current theories favor the induction of an immune response directed against specific pathogenic T-cell clones.[4] Investigative studies and clinical trials are currently under way to assess the usefulness of photopheresis in the treatment of a variety of diseases thought to be T-cell mediated including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), progressive systemic sclerosis (scleroderma or PSS), pemphigus vulgaris and organ transplant rejection.[4]

The BE2 surface marker is not detectable on the surface of normal resting peripheral blood lymphocytes, but is

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(scleroderma or PSS), [illegible]
rejection.[9]

The HB2 surface marker is not detectable on the surface of normal resting peripheral blood lymphocytes, but is expressed by a population of B cell chronic lymphocytic leukemia lymphocytes, by the EBV transformed B cell line GM 1056 and by the T-cell lines MOLT 3 and HUT 78 in addition to lymphocytes from patients with CTCL.[2] Subsequent studies of the BE2 surface marker have suggested its importance as an activation marker and have demonstrated its presence on PHA, Con A and anti-CD3 stimulated normal T-cell lines.[5] BE2 expression by peripheral blood lymphocytes has been found in 48% of patients infected with the acquired immune deficiency virus.[6] Its presence on up to 36% of the peripheral blood cells of patients with progressive systemic sclerosis has recently been discovered.[7] It has been proposed that scleroderma may be a T-cell mediated disease.[8] Both CTCL and scleroderma have prominent cutaneous manifestations.

With the cloning of the T-cell receptor, a powerful new method of identifying clonal populations of T-cells became available.[9] Gene rearrangement studies using the ß chain of the T-cell receptor as a probe have proven useful in definitively diagnosing and establishing the clonal nature of a variety of T-cell lymphoproliferative disorders.[10-15] Southern blot analysis using the ß chain of the T-cell receptor for the detection of gene rearrangements is capable of detecting clonal populations of T-cells which represent as few as 1% of peripheral blood components.[16] In CTCL this method has proven to be superior to surface marker analysis

for diagnosis, staging and prognostic predictions.[17,18]

Recent work aimed at characterizing the BE2 surface antigen marker has pointed to the value of identifying differences between BE2+ and BE2- populations of T-cells.[5] Our study was undertaken to test the hypothesis that the BE2 surface marker is a marker of clonality. We hypothesized that BE2 expression might be restricted to the malignant T-cell clone present in CTCL. In order to test this theory, we subjected genomic DNA extracted from BE2+ and BE2- peripheral blood cells from patients with CTCL to Southern blot analysis using the ß chain of the T-cell receptor to detect gene rearrangements indicative of clonal T-cell populations. In addition, we examined DNA extracted from peripheral blood cells from patients with PSS to further test the hypothesis that BE2 identifies a clonal population of T-cells by investigating whether there is a detectable clonal T-cell expansion present in the peripheral blood of patients with PSS.

CUTANEOUS T-CELL LYMPHOMA (CTCL)

CTCL is a malignant neoplasm of CD4+ lymphocytes which initially demonstrates preferential epidermal infiltration. Disease progression results in lymph node, peripheral blood and visceral involvement.[19] It is generally accepted that the designation CTCL encompasses the disorders mycosis

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CUTANEOUS T-CELL LYMPHOMA (CTCL)

CTCL is a malignant neoplasm of [illegible] initially demonstrates predilection [illegible] Disease progression results in lymph node, peripheral blood and visceral involvement.[10] It is generally accepted the the designation CTCL encompasses the disorders mycosis fungoides and Sézary syndrome.[20] Edelson has postulated that CTCL encompasses a wider variety of T-cell disorders with cutaneous manifestations including lymphoma cutis, histiocytic lymphoma and many adult T-cell leukemias.[21] In his view, each of these entities falls within a continuous spectrum of a single type of malignancy.[22] Each of these disorders is characterized by skin infiltration with neoplastic T-cells of the helper/inducer CD4+ phenotype.[23-25]

Epidemiologic investigations intending to document the precise incidence of CTCL have been complicated by the fragmentation of the disorder into various subgroups. If Edelson's broad definition is used, CTCL now appears to have a cumulative incidence equal or exceeding that of Hodgkin's disease, making it the most common adult lymphoma.[26] It occurs primarily in middle-aged white males though it may be seen in both men and women of all ages and races.[27]

The exact etiology of CTCL remains unclear. Infectious, genetic and environmental causes have been proposed.[28] Leukemia or lymphoma has been found in first degree relatives of 4% of patients with CTCL including the development of CTCL in a father and daughter.[29] HTLV-1 has been isolated from the sera of patients with adult T-cell leukemia/lymphoma, a disease in which cutaneous infiltration may be seen, but is not a feature of most cases of CTCL.[30]

Clinical Features. CTCL classically begins as flat

macules or patches (premycotic stage) which progress to papules or plaques which are slightly elevated (lichenoid stage) and then to large tumors or nodules. These stages may develop into an exfoliative erythroderma characterized by leukemic involvement. Palpable lymphadenopathy and hepatosplenomegaly reflect visceral involvement.

The term mycosis fungoides, coined by Alibert in 1806, refers to mushroom like tumors protruding from a desquamating rash.[31] This early epidermotropic phase is progressively replaced by aggressive nonepidermotropic lesions. The Sézary syndrome, first described in 1938, denotes the triad of erythroderma, leukemia composed of large mononuclear cells with convoluted nuclei, and enlarged lymph nodes.[32] Plaques infiltrating the face may result in a "leonine facies," also seen in leprosy and leishmaniasis. Lesions, which commonly present on unexposed skin areas, vary in size and color and may be asymptomatic or intensely pruritic. Plaques may range in color from red to violet. Tumor formation may occur within a preexisting plaque or may arise in noncontiguous sites. Aggressive vertical spread into the reticular dermis and subcutis is referred to as "tumor d'emblée"[33] and results in brilliantly colored lesions which occur preferentially on intertriginous areas and are predisposed to ulceration and secondary infection. In addition, these tumors are indicative of an aggressive malignant phenotype with a very poor prognosis. In patients with exfoliative erythroderma,

fissuring and scaling of the soles and palms may occur resulting in markedly diminished manual dexterity and painful ulceration. These patients often become cutaneous cripples who are handicapped physically as well as emotionally secondary to their disfigured appearance. Disseminated disease is characterized by lymphadenopathy and hepatosplenomegaly. Forty-seven percent of all patients and 80-90% of those with erythrodermic stage disease have palpable lymphadenopathy.[34] The bone marrow is uncommonly involved histologically; the absence of bone marrow involvement in the setting of leukemia is considered typical in CTCL.[34] Extracutaneous disease is often not clinically apparent but frequently found at autopsy. Survival correlates directly with the existence of extracutaneous disease. Disseminated CTCL may involve virtually any organ system. Pulmonary, cardiac, gastrointestinal, renal, central and peripheral nervous system involvement have all been reported. Extracutaneous spread of the disease spares the bone marrow until extremely late in the course of CTCL when an "acute leukemic phase" may occur.

Laboratory and Pathologic Findings. As stated above, CTCL is a malignant neoplasm of thymus-derived CD4+ lymphocytes. These cells have been shown to promote differentiation of normal B cells into immunoglobulin secreting plasma cells. This holds true for both leukemic phase cells and cells extracted from skin and lymph nodes.[24]

Immunophenotypic surface antigen studies have typically shown these cells to be CD3+, CD4+, CD8-, consistent with a "helper" phenotype.[35] However, aberrant phenotypes may be found, especially in advanced cases of the disease.[36]

Tissue distribution of the malignant cells is characteristic of the T-cell phenotype with lymph node and spleen involvement occupying the T-cell paracortex and periarteriolar sheath areas.[37] Lymph nodes do not appear to serve as barriers to spread. Karyotypic studies of cells infiltrating distinct tissue sites have demonstrated identical chromosome banding patterns strongly suggesting that CTCL is the result of a monclonal proliferation of T-cells.[38,39] No consistent karyotypic abnormality has been found in patients with CTCL though Shapiro et al found a slightly increased incidence of rearrangement of chromosome #10 which contains the gene for the interleukin-2 receptor.[40] The alpha, beta and gamma chains for the T-cell receptor are located on chromosomes 14q, 7q and 7p respectively.[41,42] Although no consistent chromosomal rearrangements involving these chromosomes have been reported, such rearrangements have been shown to occur sporadically in T-cell lymphomas. Perhaps the most definitive evidence demonstrating the clonal nature of CTCL has come from studies using the ß chain of the T-cell receptor as a probe to detect gene rearrangements.[17]

Patients with CTCL frequently have elevated serum Ig

levels, especially IgE and IgA, another finding consistent with a "helper" cell proliferation.[43] Associated monclonal gammopathies and autoantibody production have been reported.[44,45] Decreased levels of null cells have also been reported in CTCL.[46] The role these abnormalities may play in the progression of the disease and the decreased resistance to viral infections found in some patients with CTCL is unknown.

The observation that cutaneous involvement is pathognomonic of CTCL raises the possibility that malignant T-cells have an affinity for the skin. It has been hypothesized that CTCL may represent an exaggeration of the T-cell's physiologic homing mechanism to the skin.[22] Epidermal factors may be important in the epidermotropism demonstrated by CTCL. Monoclonal antibody studies have demonstrated increased epidermal interleukin-1 (IL-1) in the skin of patients with CTCL when compared with normal controls and patients with inflammatory dermatoses.[47] It is under the influence of IL-1 that T-cells produce IL-2 and proliferate.[48] This could provide a mechanism for local expansion of CTCL cells within the skin.

Diagnosis. The diagnosis of CTCL can be difficult, especially in the early stages of the disease. Interestingly, many patients who eventually received the diagnosis of CTCL previously carried the diagnosis of a variety of dermatoses

including psoriasis, chronic contact dermatitis, eczema, pityriasis rubra pilaris or atopic dermatitis. This suggests either that these conditions may predispose a patient to the development of CTCL or more likely, that the diagnosis of CTCL is difficult in its early stages.[27] The Mycosis Fungoides Cooperative Study Group found that 77% of patients previously carried the diagnosis of benign skin conditions with 32% of the diagnoses being "dermatitis not otherwise specified."[49]

Historically, evidence from the history and physical examination was combined with light microscopic examination of hematoxylin and eosin stained sections of skin biopsies to detect typical morphologic patterns. Collections of mononuclear cells in the epidermis, termed "Pautrier microabscesses" were thought to be suggestive of patch stage CTCL. This term is a misnomer, since the word abscess implies a neutrophil infiltrate, and the infiltrating cells in these lesions are lymphocytes. However, an identical picture may be seen in chronic dermatitis, making this finding nonspecific. The cellular infiltrate of plaque stage CTCL is often composed of mononuclear cells with deeply indented nuclei; as the disease progresses, the cells become more atypical in appearance. Atypical lymphoid cells with distinctive hyperconvoluted or cerebriform nuclei accumulate first in T-cell areas of lymph nodes and then infiltrate the entire node, destroying its normal architecture as the

disease progresses.[50] It is often impossible to distinguish dermatopathic lymphadenopathy from CTCL by light microscopic examination. Furthermore, patients with CTCL will often exhibit dermatopathic lymphadenopathy in addition to malignant changes, especially early on in their disease. The establishment of nodal involvement has diagnostic and therapeutic implications. Malignant lymph node involvement clearly portends a worse prognosis. It is rare for a patient to demonstrate visceral involvement in the absence of lymph node involvement.[51,52]

Examination of peripheral blood cells may reveal the characteristic serpentine nuclei of Sézary cells; however, numerous investigators have proven that the presence of these cells is not restricted to CTCL. They may also be found in psoriasis, lichen planus and in benign cutaneous conditions.[53] In addition, the existence of nonneoplastic circulating Sézary-like cells, thought to represent reactive lymphocytes, has been documented in patients with CTCL.[54] The ultrastructural features of these distinctive cells by electron microscopy have also proven not to be specific for CTCL.[55]

DNA cytophotometry which quantitates abnormalities of nuclear DNA content, and cytogenetic (karyotypic analysis) studies have also been used to diagnose CTCL. While karyotypic analysis is more sensitive than DNA cytophotometry, both methods are technically difficult to

perform and have variable specificity. Immunophenotypic analysis has a limited but useful role in distinguishing CTCL from other lymphoid disorders.[56] The detection of clonal rearrangements of the T-cell receptor genes by Southern blot analysis, which was instrumental in determining the clonal nature of this disorder, may be the most definitive diagnostic test for patients with CTCL. The method is substantially more sensitive than histologic examination of lymph node specimens for the detection of occult disease.[17] It also establishes an effective test useful for assessing the peripheral blood of these patients.[18] The utility of this technique has not been extended to the diagnosis of plaque or early stage disease. Ralfkiaer et al found no evidence of a monoclonal population in skin biopsies from 12 patients, 8 of whom had histologically nondiagnostic, but clinically suspicious, lesions and 4 of whom had stage II mycosis fungoides.[57]

A combination of the aforementioned diagnostic techniques currently provides the most complete assessment of a patient's disease involvement. Patients with CTCL are staged according to a modified TNM system.[58] Peripheral blood involvement is not incorporated into the TNM classification; it is thought to be a separate prognostic factor which may correlate with total body tumor burden.[59]

Mycosis fungoides, unlike Hodgkin's lymphoma, does not peculiar [illegible] etiology [illegible] [illegible] measurement [illegible] and [illegible] active [illegible] [illegible] [illegible] [illegible] lymph [illegible] [illegible] [illegible] [illegible] particular [illegible] clinical [illegible] mycosis fungoides.

[illegible] techniques [illegible] a patient's [illegible] staged according to a modified TNM [illegible] blood involvement [illegible] classifications; it is thought [illegible] factor which may correlate with total body tumor burden.[19]

Mycosis fungoides, unlike Hodgkin's lymphoma, does not progress in an orderly fashion from one chain of lymph nodes to the next. Prospective studies strongly indicate that CTCL spreads hematogenously rather than by extension via the lymphatics. Once the malignant cells invade beyond the confines of the skin, the lymph nodes do not provide a barrier to further spread. Thus, widespread dissemination is to be expected in patients with evidence of lymph node involvement.[49]

Treatment. The choice of appropriate treatment depends on accurate staging. Early patch or plaque stage lesions may be successfully treated with topical or intralesional corticosteroids.[60] Topical nitrogen mustard or mechlorethamine may also effectively control limited cutaneous disease as will carmustine or bischloroethyl-nitrosurea (BCNU).[61,62] Patients with purely cutaneous involvement may show dramatic improvement following treatment with 8-methoxypsoralen plus ultraviolet A light (PUVA)[63,64], a therapy first proven to be effective in the treatment of psoriasis.[65]

Eight-methoxypsoralen (8-MOP) is absorbed orally. Peak plasma levels are reached within two to three hours. In the presence of UVA light 8-MOP forms covalent DNA photoadducts with pyrimidine bases.[66] Only 60% of UVA light penetrates to the dermis and less than 1% to the subcutis. Thus, this therapy does not affect deep tumor infiltrates or sites of

visceral involvement.[63] PUVA must be given 2-3 times per week until intial skin clearing occurs at which time maintenance therapy is required. Electron-beam treatment may also be useful in CTCL confined to the skin.[67] Leukapheresis may provide palliative therapy for patients with erythrodermic stage CTCL.[68]

Each of the above treatments is effective for patch or plaque stage CTCL without lymph node, visceral or leukemic involvement. Systemic chemotherapy is merely palliative for advanced disease.[69] However, occult systemic involvement, common in advanced disease, may exist at the time of presentation.[70] A recently completed randomized trial compared combination electron-beam radiation and chemotherapy to topical treatment. The results showed no advantage of aggressive initial treatment in comparison to conservative treatment.[71]

Prognosis. CTCL carries a prognosis which clearly depends on disease stage at the time of diagnosis, since effective therapies exist for disease limited to the skin. In a retrospective study, Epstein et al analyzed 144 patients at the National Cancer Institute, all of whom had skin biopsy findings consistent with mycosis fungoides.[72] Mean survival after the appearance of skin lesions was 8.8 years, with the mean survival from the time of histologic diagnosis being less than 5 years. The presence of palpable lymphadenopathy

or cutaneous tumors reduced mean survival to 2.5 years, and patients with hepatomegaly and/or splenomegaly had a mean survival of only 3 months.

Lamberg et al identified several other prognostic factors. Plaque, papule or patch stage involvement covering more than 10% of the total body surface area correlates with a poorer prognosis, as does generalized erythroderma and/or cutaneous tumors.[49] Individuals with erythrodermic CTCL have a median survival less than 30 months. Patients with CTCL usually die from infectious complications resulting from their underlying disease, from therapy, or from complications of involvement of vital organs.[72]

EXTRACORPOREAL PHOTOCHEMOTHERAPY (PHOTOPHERESIS)

Dramatic progress in the treatment of CTCL occurred when Edelson et al developed a new treatment for systemically disseminated disease. As previously mentioned, PUVA, in which a patient's skin is exposed to UVA light following the oral administration of methoxsalen has proven efficacy in plaque stage CTCL.[63,64] This knowledge, combined with the fact that leukapheresis provided effective treatment for some patients prompted Edelson to test the hypothesis that exposing leukocytes to the effects of PUVA might be efficacious for leukemic phase disease. Since neither platelets nor red blood cells contain nuclei, they would not be affected by the treatment and could be returned to the

patient. Returning these cells to the patient would prevent the occurence of anemia and thrombocytopenia, limiting side effects of leukapheresis. His hypothesis evolved into an effective treatment known as photopheresis.[73]

In the initial multicenter trial of photopheresis, 41 patients were treated. Thirty-three had generalized erythroderma, and 8 had localized plaques. Each patient had circulating atypical mononuclear cells and a diagnostic skin biopsy. After ingesting .6 mg/kg body weight of methoxsalen, 240 mls of leukocyte enriched blood containing 30-50% of the patient's circulating lymphocytes were passed as a 1 mm film through a sterile cassette exposed to UVA light at 1-2 joules per square cm prior to being returned to the patient.

T-cell subsets using monoclonal antibodies were determined for each patient. Additionally, reactivity with BE2 was tested. Lymphocytes from 4 patients in the study were exposed to 8-MOP and UVA light but not returned to the patient so that the viability of these cells following treatment could be determined. Seventy-two hours after treatment only 12 +/- 5% of these lymphocytes were viable as determined by trypan blue exclusion. The author further noted that more than 25% of the lymphocytes from these patients were BE2+. This observation suggested that if reactivity with the BE2 antibody is specific for the malignant clonal population, then the levels of 8-MOP and UVA utilized were sufficient to kill tumor as well as normal

lymphocytes.

Of the initial 41 patients, 37 completed the trial. All had failed prior therapy, which included systemic chemotherapy, electron-beam radiotherapy, topical mechlorethamine, or PUVA directed at the skin. Side effects typically seen with systemic chemotherapy such as alopecia, bone marrow suppression and GI erosions did not occur. Notably, a post-reinfusion febrile spike occurred in four patients, all of whom ultimately responded to treatment. Twenty-seven of these patients responded to treatment. Of the 28 patients who had failed systemic chemotherapy, 20 responded to photopheresis. Twenty-four of 29 patients with exfoliative erythroderma responded while 3 of 8 with localized plaques and tumors showed a positive response. Response was defined as a 25% reduction in the baseline overall skin lesion score maintained for at least 4 consecutive weeks. Nine patients showed more than 75% response, 13 showed 50-75% response and 5 a 25-49% response.

Eleven of the patients were shown to carry clonal populations of T-cells as determined by T-cell gene rearrangement studies or karyotypic analysis. No consistent changes in total leukocyte counts, lymphocyte percentages or the number of circulating Sézary cells were found. However, when the 11 patients with clonal T-cell populations were analyzed individually, the following observations were noted. Six of these patients initially had >90% CD4+ cells with 3

patients demonstrating higher percentages of CD4+ cells than CD3+ cells, possibly indicating abnormally deficient CD3 expression by these malignant cells. During the course of treatment, the CD4/CD3 ratio progressively decreased in these patients.

Of particular interest to us, was the fact that in the 5 subjects who had initially elevated BE2+ levels all exhibited a significant decrement in BE2 upon achievement of a clinical response. The author suggested that the BE2 marker distinguishes the malignant T-cells of some patients from their normal helper/inducer T-cells and permitted a laboratory correlation of the response to therapy. In addition, those patients who experienced a substantial decrease in the percentages of their cells expressing the BE2 marker after treatment, also exhibited a continued decline of the BE2 population. Levels remained very low for several months. In 2 patients the decrement persisted for more than 2 years, an observation which correlated with quiescent disease activity.

The exact mechanism of photopheresis remains elusive. The photodestruction of large numbers of T-cells belonging to an expanded clone may, upon introduction to an intact reticuloendothelial system, provoke a specific immunologic reaction directed against the expanded clone. Experiments in animal systems have shown that the injection of specific autoreactive clones of T-cells can induce a variety of

autoimmune syndromes including encephalomyelitis, arthritis and thyroiditis. Experimental autoimmune encephalomyelitis (EAE), adjuvant arthritis (AA) and experimental autoimmune thyroiditis (EAT) are all T-cell mediated organ specific diseases.[74] Investigators have also demonstrated, however, that the infusion of lethally damaged specific autoreactive T-cell clones leads to resistance to autoimmune disease upon subsequent infusion of viable pathogenic T-cell clones.[75,76] Resistance to subsequent induction of autoimmune disease has been attributed to a specific clonotypic immune activation, perhaps via the induction of specific suppressor-inducer T lymphocytes. Cohen refers to this phenomenon as "T-cell vaccination." Vaccination is achieved by treating the cells with hydrostatic pressure or with chemical crosslinkers such as formaldehyde or glutaraldehyde that crosslink cell membrane components.[77] These methods are effective in preventing disease though they do not affect already established disease.

Other groups have recently shown that exposure of cells to 8-MOP/UVA may produce a "vaccination" process identical to other methods used by Cohen and his group. This is particularly significant because hydrostatic pressure and formaldehyde are not treatments which could easily be adopted for use in human subjects. However, numerous clinical trials have shown photopheresis to be remarkably free of limiting side effects.[3]

Khavari et al have shown that photoinactivation of cells with 8-MOP/UVA effectively protects against the development of EAE.[78] Perez has similarly demonstrated that these cells, termed "photoinactivated effector lymphocytes" can induce tolerance for skin allotransplantation in murine models.[79] This method has also proved to be effective in preventing a murine SLE-like syndrome related to an unregulated proliferation of benign aberrant inducer T-cells.[80,81] Berger has recently shown in animal models that photoinactivated spleen cells show, upon reinjection, increased sequestration by the bone marrow, spleen, and kidney - sites where an immune response may be induced. Furthermore, photopheresis added to conventional immunosuppressive regimens has prolonged graft survival, suppressed the mixed leukocyte culture response to the donor, and suppressed antidonor antibody formation in a primate cardiac xenograft model.[82]

While 8-MOP/UVA is thought to exert its effect at the DNA level via pyrimidine crosslinking,[66] the identification of high affinity binding sites on the cell surface raises the possibility of a mechanism of psoralen toxicity not involving DNA intercalation.[83] Photopheresis is not specifically targetted at malignant or aberrant T-cell clones, but expanded T-cell populations are probably damaged more extensively than other cells. This might result in selective

destruction of the malignant clone by intact cells of the immune system not exposed to the damaging effects of 8-MOP/UVA.[73] Photopheresis has received FDA approval for the treatment of advanced CTCL.[84] In accordance with this principle, extensive clinical experience involving photopheresis for the treatment of CTCL has shown that immunocompetent patients identified by PHA stimulation assays responded best.[85] Patients who are not immunocompetent may respond to frequent treatments, but this response is thought to result from simple physical destruction of malignant cells and is not long-lived.[86]

The possibility that exposing nonfractionated peripheral blood lymphocytes to 8-MOP/UVA treatment can result in a specific clonotypic response has particularly attractive therapeutic implications. Photopheresis does not require isolation of specific aberrant clones, a feat which has not yet been possible. In addition, as already stated, the treatment has minimal systemic toxicity. Currently, clinical trials are underway to investigate the efficacy of photopheresis in a variety of diseases including autoimmune disorders such as SLE, RA, PSS and pemphigus vulgaris. A clinical trial involving patients with chronic lymphocytic lymphoma (CLL) is also in progress, and there perhaps lies a role for photopheresis in the area of organ transplantation.[87,88] Preliminary results in patients with

pemphigus vulgaris have been striking.[89] Clinical trials in CLL and discordant xenograft protocols in which preformed antibodies exist, should help elucidate the effect of photopheresis on B cells.

BE2

BE2 is a murine monoclonal antibody produced by immunizing mice with leukemic helper T-cells from a patient with CTCL.[2] When purified, the BE2 antibody precipitated a single 78,000 D molecule from CTCL cells. Reactivity was determined by indirect immunofluorescence and immunoperoxidase staining. By these methods BE2 reacted strongly with CTCL cells. Ten percent reactivity, which was two standard deviations above normal background, was chosen as the cutoff for significant reactivity.

Cells from 5 of 8 patients with B cell CLL reacted with BE2. The antibody also reacted with lymphocytes from one patient with leukemic CTCL and with lymphocytes from a lymph node of a patient with non-leukemic CTCL. It reacted with lymphocytic infiltrates in skin biopsy and lymph node specimens in patients with advanced CTCL. Normal lymph nodes, uninvolved epidermis and thymus sections did not react with BE2 on the basis of immunoperoxidase staining. Normal tissues screened included thyroid, skin, breast, thymus, colon, liver, kidney, lung, heart, brain and lymph node. BE2 showed strong affinity for EBV transformed B cell lines by

radioimmunoassay. The long term cell lines MOLT 3 and HUT 78 also reacted with BE2. The antibody did not react with four melanoma cells lines, two breast cancer lines, a colon cancer line and promyelocytic, myelocytic, erythroid, or null cell lines.

Twenty-one patients with clinical CTCL, without apparent lymph node involvement were tested for peripheral blood reactivity to BE2. Five patients (Group I) were subsequently found to have disseminated disease by lymph node biopsy and karyotypic analysis. Four of these five patients showed >10% reactivity with BE2. Every patient with proven extracutaneous disease had peripheral blood cells reactive with BE2. Eight patients (Group II) had widespread erythroderma. Five of these patients reacted with BE2. Of note, 3 patients with advanced disease did not react with the BE2 antibody. Group III consisted of another 8 patients with evidence of disseminated disease. Four of these patients reacted with BE2; less than 10% of the circulating mononuclear cells from the other 4 patients reacted with BE2. Thus, 62% or 13 of 21 patients demonstrated reactivity to BE2. Ten of these patients had no clinical evidence of extracutaneous disease.

Overall, 8 patients did not demonstrate reactivity with BE2, with 3 of these patients having evidence of disseminated disease. This latter observation led the authors to hypothesize that patients with end stage aggressive disease

may lose the BE2 antigen. A more likely explanation would be that BE2 is simply not expressed on all CTCL cells. The authors postulated that reactivity with the antibody BE2 signified the presence of extracutaneous disease. However, only 4 of these patients had evidence of extracutaneous disease on the basis of lymph node biopsy or karyotypic analysis. Importantly, 61% of the reactive patients had no clinical evidence of disseminated disease and were suspected of having extracutaneous disease solely on the basis of reactivity with this antibody.

PHA stimulated mononuclear cells from normal controls and purified normal B cells also did not demonstrate reactivity. Patients with widespread psoriasis and normal controls were shown to be nonreactive with BE2.

In summary, Berger et al concluded that BE2 is present on several transformed T and B-cell lines in addition to lymphocytes from patients with CTCL. They further concluded that the absence of BE2 expression by normal lymphocytes and normal tissue suggested that its expression is restricted to neoplastic cells of lymphoid origin; they further postulated that BE2 expression might therefore be useful in identifying small numbers of neoplastic cells. Finally, they propose that further studies aimed at isolating and characterizing the BE2 antigen should reveal its functional role. They also suggest that if BE2 is a specific tumor-associated antigen, further studies might clear new pathways for therapy of CTCL.

Berger's group extended this work by studying the *in situ* cutaneous infiltrate of 5 patients with digitate dermatosis, 12 with pityriasis lichenoides chronica, 3 patients with lymphomatoid papulosis and 3 with cutaneous B cell lymphoma. In the same study 19 patients with CTCL were reactive with the BE2 marker. Among the other dermatoses investigated only one patient with lymphomatoid papulosis and one patient with cutaneous B cell lymphoma reacted with BE2. From this study the authors concluded that the BE2 antigen allows unambiguous tissue diagnosis of CTCL.[90] Another group reported that patients with actinic retinoid failed to react with BE2.[91]

In contradiction to Berger's results, Ralfkiaer et al concluded that BE2 is neither T-cell nor tumor specific.[92] Ralfkiaer et al examined a comprehensive range of nodal and cutaneous biopsies immunohistologically using single and double immunofluorescent staining methods. BE2 demonstrated an extremely broad range of reactivity. It consistently labelled normal endothelial cells, mantle and marginal zone B cells, and T-cells in the lymph node paracortex and thymic medulla. It also labelled Langerhans cells and dermal macrophages. Seventeen of 28 cases of malignant T-cell lymphoma studied showed reactivity with BE2. T-cells from 4 of 19 benign dermatoses also reacted with BE2. Clearly, the antibody reacted in a nonspecific fashion.

The authors employed a multistage staining method. For

double staining, sections were incubated with a mixture of anti-BE2 antibody, an IgGI antibody, and anti-LeuI, an IgG2a antibody, followed by staining with fluorescein and rhodamine conjugated rabbit antibodies against mouse IgGI or IgG2a immunoglobulin subclasses. Omission of the primary monoclonal antibody, substitution of an IgG2a reagent for the IgGI antibody and similar substitution of an IgGI reagent for an IgG2a reagent were used as negative controls. Substitution of secondary antibody should not be used as a negative control. Ideally, negative controls should be stained under identical conditions with identical reagents on a known negative population of cells. The lack of appropriate controls in this study renders their conclusions ambiguous.

Berger et al later demonstrated that 48% of patients with the acquired immunodeficiency syndrome (AIDS) had peripheral blood populations which reacted with BE2.[6] Interestingly, the HTLV-I virus, a member of the retrovirus family which includes the AIDS virus, has been isolated from the CTCL patient whose lymphocytes were used to immunize mice to produce the BE2 antibody.[30]

It would be interesting to know how many of the AIDS patients in the study had skin involvement with Kaposi's sarcoma or other cutaneous disorders. The authors did state that AIDS patients with and without Kaposi's sarcoma were among the BE2+ population. The authors propose that BE2 may

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sarcoma or other cutaneous abnormalities [illegible]
that AIDS patients with and without [illegible] were
among the HIV+ population. The authors propose that PTI may

be a normal cellular product whose expression can be induced by retroviral infection. Humoral titers of HTLV-I were not elevated in the BE2+ AIDS population. The previous demonstration of BE2 expression by an EBV transformed B cell line makes the restriction of BE2+ expression to T-cells in AIDS patients of interest. Finally, the authors propose that the BE2 antigen may be restricted to a small subset of normal T-cells which have been expanded in response to a chronic antigenic challenge.

Recently, induction of BE2 expression by normal T-cells and by BE2- CTCL cells has been reported.[5] Induction followed stimulation of the cells with PHA, Con A, allogeneic leukocytes, and anti-CD3 monoclonal antibody. It was also noted that BE2 antibody inhibits PHA induced thymidine incorporation and IL-2 expression, leading investigators to conclude that the antigen has a role in T-cell activation. Thus, in contradiction to earlier suggestions, the authors assert that the BE2 antigen may be a functional membrane protein and that its expression may be a normal physiologic process which is altered in CTCL.

BE2 is proposed to be a late activation antigen which directly contributes to cell proliferation. According to this hypothesis, CTCL patients may be divided into those whose cells constitutively express BE2 and those in whose cells expression can be induced. The authors also imply that photopheresis is more effective in CTCL patients with be a normal cellu[illegible]

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photopheresis is more effective in CTCL patients with circulating BE2+ cells. The presence of BE2 antigen expression may reflect activated circulating malignant cells; these may be necessary for the induction of a T-cell specific clonotypic response.

In summary, BE2 is a murine monoclonal antibody raised against malignant CD4+ cells from a patient with CTCL. It reacts with approximately 75% of the peripheral blood cells of patients with CTCL. It does not react with peripheral blood cells from normal controls, but is detectible in 48% of patients infected with AIDS. In addition, it reacts with B cell CLL lymphocytes, an EBV transformed B cell line and two T-cell lines. Induction of BE2 expression on normal T-cells and PHA, Con A and anti-CD3 stimulated BE2- cells has suggested its importance as an activation antigen.

PROGRESSIVE SYSTEMIC SCLEROSIS (PSS OR SCLERODERMA)

The recent revelation that patients with PSS constitutively express BE2[7] provided us with an ideal population of patients in whom to test the hypothesis that BE2 expression is restricted to either a malignant T-cell clone or an expanded and perhaps autoreactive clone. In addition, the documentation of a detectable T-cell clone in patients with PSS could have important therapeutic implications with respect to the usefulness of photopheresis in PSS.

Progressive systemic sclerosis is a generalized

disorder of unknown etiology characterized by excessive connective tissue accumulation. The classic finding in the disease is induration or thickening of the skin (scleroderma). The disease is also characterized by systemic fibrosis of the heart, lungs, kidneys, gastrointestinal tract, muscles and synovium. Vascular and microvascular abnormalities are prominent features of the disease. The characteristics of PSS, which have been reviewed thoroughly,[93] will be summarized briefly here.

Epidemiology. Systemic scleroderma is usually diagnosed in the fifth or sixth decade. Its distribution is global with blacks being affected more often than whites and females being affected approximately three times as often as males. Clear occupational and environmental exposure risks have been identified with silica dust being of particular note. Numerous studies have suggested a heritable component to the disease. More than 25% of blood relatives have +ANA titers compared to 5-8% of controls; however, monozygotic twin studies do not point toward a clear genetic predisposition, and family studies have not established a predominant HLA haplotype. Chromosomal instability, observed in spontaneous mitoses from bone marrow, has been reported. An increased incidence of random chromosomal breakage has also been described in patients with PSS and their asymptomatic relatives.

Clinical Features. Patients with limited cutaneous

disease often present with an initial complaint of Raynaud's phenomenon while patients who go on to develop diffuse scleroderma often present with skin tightening or arthritis. In rare patients, internal manifestations are the first sign, or internal disease may exist in the absence of cutaneous involvement.

Cutaneous involvement usually progresses from an initial edematous phase to an indurative phase which may eventually soften or enter an atrophic phase. Histologically, accumulations of mononuclear cells, mostly T lymphocytes, are found in the lower dermis and subcutis. Mast cells are often found in early disease. There is also a marked increase in the number of compact collagen fibers in the reticular dermis, loss of rete pegs, and thickening of the epidermis. Areas of hyper- and hypopigmentation occur over the thickened skin creating a "salt and pepper" appearance. Intra- and subcutaneous calcifications may form, often on digital pads and periarticular tissues, and along extensor surfaces and bursal areas. Ulcerations and secondary bacterial infections may complicate the clinical picture.

Ninety-five percent of patients with PSS have Raynaud's phenomenon. Narrowing and obstruction of the digital arteries can be demonstrated. Patients frequently complain of polyarthralgias and joint stiffness. A predominant mononuclear infiltrate may be found in involved large joints disease [illegible]

[illegible]

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monoarticular inflammation may be found in involved large joints and inflammatory cells are also found in the synovium. Tendinous and periarticular fibrosis results in joint contractures. The terminal phalanges may demonstrate erosive arthropathy. Muscle atrophy may result from disuse or may be secondary to myopathy from inflammation or indolent connective tissue fibrosis. Muscle biopsies show focal collections of plasma cells and lymphocytes mostly in perivascular areas.

Gastrointestinal involvement is a significant cause of morbidity. Microstomia and microchelia are often present, and thickening of the periodontal membrane and loss of the lamina may result in loosening of the teeth and gingivitis.

Ninety percent of patients develop esophageal dysfunction, the most common internal manifestation. Symptoms of dysphagia often precede cutaneous disease. Incompetence of the lower esophageal spincter results in gastroesophageal reflux and esophagitis. Smooth muscle atrophy and fibrosis and lymphocyte and plasma cell infiltration of the lamina propria are seen histologically. These patients have a predisposition for the development of Barrett's esophagus.

Gastric involvement may result in delayed emptying. Abdominal pain, nausea, vomiting, weight loss and bloating are symptoms which reflect small intestine involvement. Malabsorption and vitamin B12 deficiency result from bacterial overgrowth. Histologic changes are identical to

those seen in the esophagus. Such changes may also exist in the colon, resulting in diarrhea, constipation or obstipation. Wide neck diverticula, usually on the antimesenteric border of the transverse and descending colon, are unique to scleroderma.

Primary biliary cirrhosis has been seen in patients with scleroderma, particularly those who also have Sjogren's syndrome. These patients have antimitochondrial antibodies, usually directed against a 72,000 D M2 autoantigen.

The respiratory system is affected in over 70% of patients with scleroderma. Exertional dyspnea is the most commmon symptom, often accompanied by a dry, nonproductive cough. Bibasilar crackles are often heard on physical exam, and chest radiographs often demonstrate diffuse linear and nodular fibrosis. PFT's are also diffusely abnormal with a reduced diffusing capacity being characteristic. Patients generally experience a progressive deterioration in pulmonary function. Idiopathic pulmonary fibrosis with alveolar and interstitial fibrosis is the histopathological finding. Loss of septa may lead to honey-combing. In early disease alveolitis with infiltration by lymphocytes, macrophages, neutrophils and eosinophils is seen. Many investigators feel that lung fibroblasts are stimulated by mononuclear cell products.

Cardiovascular manifestations of scleroderma consist primarily of pericarditis, left ventricular or biventricular

congestive failure or arrrythmias. Diffuse atrophy and myocardial fibrosis are the histopathologic changes seen and are thought to occur secondary to vasomotor and mirocirculatory dysfunction (myocardial Raynaud's phenomenon). Coronary artery disease does not occur with greater frequency in patients with scleroderma.

Renal involvement is a prominent internal manifestation in scleroderma and a major cause of mortality in these patients. Clinical features range from asymptomatic proteinuria to scleroderma renal crisis (malignant arterial hypertension, pulmonary edema and renal failure). Renal blood flow abnormalities include decreased renal plasma flow and a reduction in renal cortical blood flow. Constriction of the interlobular arteries and afferent arterioles is seen. Twenty-five percent of patients develop hypertension. Angiotensin converting enzyme inhibitors have markedly decreased hypertension associated mortality. Immunoglobulin, fibrinogen and complement deposition, with IgM being the major immunoglobulin present, are seen. Electron microscopic studies have not revealed the presence of immune complexes.

Anemia may occur late in the course of disease, but hematologic abnormalities are uncommon. Primary CNS involvement is unusual; trigeminal neuralgia is a rare presenting complaint and is associated with anti-RNP antibodies. Cerebral arteritis has been reported. In males, impotence may result from reduced penile blood flow.

Keratoconjunctivitis sicca, often seen in association with Sjogren's syndrome, is the primary ocular manifestation. Hypothyroidism occurs in up to 25% of patients and is associated with antithyroid antibodies. Sjogren's syndrome with lymphocytic infiltration of the minor salivary glands is frequently seen in patients with scleroderma.

Prognosis. Systemic sclerosis runs a variable course. The presence of cutaneous thickening prior to the onset of Raynaud's phenomenon, rapid disease progression with proximal extremity involvement, serum anti Scl-70 antibody and palpable tendon friction rubs all herald a poor prognosis. Late in the disease, skin may spontaneously soften as other manifestations also subside. Most patients, however, progress to visceral involvement. Male gender, advanced age, and renal, cardiac or pulmonary involvement are all poor prognostic factors.

Pathogenesis. Although the pathogenic mechanism in PSS remains unclear, clear vascular and immunologic abnormalities are associated with the overproduction of connective tissue proteins and collagen. Excess production of collagen is the hallmark of scleroderma. Skin biopsy explants from patients with scleroderma produce excess amounts of collagen and glycosaminoglycans, particularly hyaluronic acid and collagen. Increased levels of proline hydroxylase and lysosyme hydroxylase, enzymes involved in collagen metabolism occur. Normal fibroblasts incubated with

serum from patients with PSS demonstrate increased proliferation. It appears that fibroblasts producing high levels of collagen, perhaps a subpopulation of cells, selectively proliferate.

Clinical and histopathological evidence of vascular abnormalities in scleroderma is abundant. It has been proposed that the vascular endothelium is the major target organ. Concentric intimal hypertrophy occurs and the adventitia becomes surrounded by fibrotic tissue. The microvasculature shows endothelial swelling, duplication of the capillary basement membrane, and loss of normal capillaries. Elevation of a number of endothelial and platelet factors has been demonstrated, including von Willebrand factor, VIII-von Willebrand antigen, circulating platelet aggregates and beta-thromboglobulin. Increased thromboxane and prostacyclin synthesis has been reported. Ultrastructural examination of the endothelium of patients with PSS reveals platelets adherent to the basement membrane in damaged endothelial cell gaps. Platelet-derived growth factor (PDGF), released by activated platelets, is chemotactic and mitogenic for fibroblasts, and may have a role in the pathogenesis of scleroderma by inducing intimal proliferation. Connective tissue activating peptide-II (low affinity platelet factor 4), shown to be mitogenic for human fibroblasts, may also be involved. Abnormalities of serotonin metabolism have also been hypothesized to play a

pathogenic role.

Multiple immunologic abnormalities are found in patients with scleroderma, suggesting a central role for the immune system in the pathogenesis of the disease. The coexistence of scleroderma with other collagen vascular disorders, such as rheumatoid arthritis, Sjogren's syndrome, and autoimmune thyroiditis, provides clinical evidence of its association with classic immune mediated conditions. Additional indirect evidence also comes from the scleroderma-like skin changes which are seen in graft-versus-host disease (GVHD), a syndrome which is clearly immunologically mediated.

Abnormalities of both humoral and cellular immunity have been identified in scleroderma. Hypergammaglobulinema, typically elevated IgG levels, is found in greater than a third of patients. The existence of monoclonal gammopathies has been reported. Circulating immune complexes are sometimes detected in small quantities, primarily in patients wih detectable autoantibodies. Up to one third of patients with PSS are positive for Rheumatoid factor. Uncommonly, LE cells can be demonstrated, and rare patients have +VDRL titers.

More than 90% of patients have circulating antinuclear antibodies although titers are lower than those usually found in SLE. Nuclear staining patterns observed include fine diffuse speckles, larger discrete speckles, centromere and

nucleolar staining. The fine, diffuse speckled pattern correlates with antibodies to the Scl-70 antigen, a nonhistone nuclear protein now known to be the nuclear enzyme DNA topoisomerase I. Although only about one-fifth of patients with scleroderma are positive for the Scl-70 antibody, the marker is highly specific. Anticentromere antibodies occur most commonly in patients with limited scleroderma with 50-96% of patients with the CREST variant possessing these antibodies.

Antinucleolar antibodies are common in patients with Sjogren's syndrome in addition to PSS. A variety of other antibodies including anticentriole and anti-ssRNA antibodies have been reported. Antilymphocyte antibodies are frequently present. Antibody to type I and type IV (basement membrane) collagen have been correlated with a reduced pulmonary diffusion capacity.

Numerous abnormalities of cellular immunity have been reported in patients with scleroderma. Cellular infiltrates, primarily T lymphocytes, but also collections of plasma cells, can be detected in the skin, synovium, lung, GI tract and other locations early in the disease course. Levels of T-cells in the peripheral blood are often decreased. There is no typical change in the number of B cells. Hughes et al hypothesized that this reduction in peripheral blood T-cells is secondary to tissue sequestration. As stated above, skin infiltrates consist primarily of T-cells. Surface marker

studies have revealed an elevated CD4+/CD8+ ratio secondary to a decrease in circulating CD8+ cells in approximately one third of patients. Others have attributed the altered ratio to increased helper T-cell populations.

Recently, the role of lymphokines and monokines in the pathogenesis of connective tissue disease has been investigated. Lymphocytes from patients with PSS, when stimulated by normal human collagen or animal collagen extracts, produce leukocyte chemotactic factor and leukocyte migration inhibition factor. This raises the possibility that persistent fibroblast stimulation secondary to humoral factors may play a role in the severe fibrosis which characterizes PSS. When stimulated with mitogens, lymphocytes from patients with PSS produce a factor which stimulates human dermal fibroblasts. Lymphocyte mediated fibroblast proliferation and collagen production have been reported by several groups, but others have shown collagen synthesis to be suppressed by mononuclear cell supernatants. Jimenez has reported the inhibition of collagen synthesis of scleroderma fibroblasts by interferons.[94]

A possible role for IL-1 in the pathogenesis of PSS has been proposed based on the knowledge that IL-1 can induce fibroblast proliferation. Others have suggested that IL-1 may stimulate fibroblasts to produce collagenase. Both increased and decreased IL-1 production by mononuclear cells in PSS have been reported. These conflicting reports may be

the result of varying laboratory techniques and fibroblast culture lines as explained by Korn et al.

In a recent review, Claman proposes a key role for the mast cell in PSS.[95] Its role in the pathogenesis of PSS was first highlighted when he and colleagues working on rats with chronic GVHD noticed the absence of mast cells in the skin of these mice. It was later shown that these mast cells had degranulated rather than disappeared, rendering them undetectable by standard toluidine dye. Later studies on skin from patients with PSS showed highly activated mast cells.

The clinical observation that many patients with PSS and morphea experience pruritis at involved skin sites suggested the presence of histamine release by mast cells. Endothelial changes present in scleroderma parallel those produced by histamine. The number of mast cells is increased preceding the ingrowth of new capillaries in corneal graft rejection. It is also known that T-cell derived growth factors are capable of activating endothelial cells resulting in the changes seen in PSS. Heparin, a substance released from mast cells, and other heparin-binding growth factors are potent angiogenic stimuli. They also mediate endothelial cell and fibroblast proliferation. Other studies have linked mast cells and fibroblasts via T-cells.

In summary, Claman proposes an integrated theory of scleroderma in which endothelial cells, mast cells and

fibroblasts, all resident cells which regulate connective tissue structure and vascular tone (mast cells and endothelial cells act on the fibroblast via heparin binding growth factors) are activated by invading T-cells, macrophages and platelets, all of which secrete a variety of factors. Macrophages secrete histamine releasing factors and IL-1. T-cells release the mast cell activators IL-3 and IL-4 and also fibroblast growth factors. Claman concludes that it is unlikely that a single factor is responsible for the myriad abnormalities seen in PSS, but he emphasizes the need to incorporate the mast cell into any comprehensive theory highlighting it as a target for future therapeutic interventions.

Treatment. Treatment for patients with PSS remains inadequate. At present, therapy for PSS consists of supportive measures: calcium channel blockers for the control of Raynaud's phenomenon, lanolin based creams for skin dryness, physical therapy for contractures, NSAIDS and occasional corticosteroids for synovitis and myositis, conventional diuretics, digoxin and afterload reducers for CHF. Metoclopramide improves esophageal motility for some patients. Proper oral hygiene and measures such as small meals and esophageal dilatation provide symptomatic relief of GI involvement. Aggressive control of hypertension has successfully reduced the mortality from scleroderma renal crisis with associated malignant hypertension. The

angiotensin converting enzyme inhibitors are the mainstay of antihypertensive therapy for patients with PSS.

No single agent slows the disease process effectively. D-penicillamine, a drug which interferes with collagen cross-linking, may be effective in some patients; however, its side effects frequently mandate discontinuation of the drug. Colchicine, known to inhibit microtubule function has also been advocated, but adequate randomized double-blind clinical trials have not been carried out for either of these drugs. Corticosteroids are effective in patients with myositis or acute pericarditis, but their use in general has been disappointing. Results with immunosuppressive drugs have been variable. Plasmapheresis has resulted in similarly variable results. Controlled trials are urgently needed to evaluate the efficacy of the growing number of immunosuppressive agents available. Currently a multicenter randominized clinical trial of D-penicillamine versus photopheresis is underway.

The considerable experience with photopheresis in the treatment of CTCL has spawned numerous clinical trials to evaluate the use of this treatment for a variety of diseases thought to be T-cell mediated including PSS. Multiple immunologic abnormalities are present in PSS. The finding of lymphocytes in the skin of patients with PSS is particularly notable.[8] CTCL and PSS are both systemic diseases with cutaneous manifestations. T-cell abnormalities are prominent

in both conditions. Furthermore, peripheral blood cells from patients with PSS are reactive with BE2.

GENE REARRANGEMENTS

Rapid advances in molecular biology provided the tools which enabled scientists to elucidate the mechanisms by which antibody diversity is generated.[96] Because of the cloning of the T-cell receptor genes, investigators have been able to demonstrate that these genes rearrange in order to generate diversity in a manner analogous to the B cell immunoglobulin genes.[97] These rearrangements are detectable by Southern blotting techniques using cloned DNA fragments as molecular probes.

This technology first became useful in the clinical setting as a means for definitively demonstrating the clonal nature of neoplasms of B cell lineage. Immunoglobulin gene rearrangement analysis is capable of detecting monoclonal B cell populations which comprise as little as 1% of the total cell population.[98] This method thus provides a powerful and sensitive means of establishing the diagnosis of B cell lymphoma and leukemia in patients with confusing histology or when tumor cells lack distinctive antigenic markers. It has also been used to show that B cell lymphomas exist which are in fact biclonal.[99]

With the cloning of the genes for the T-cell receptor, this approach was extended to the diagnosis of a variety of

T-cell lymphoproliferative disorders. The human T-cell receptor consists of a disulfide-linked heterodimer composed of alpha and beta subunits weighing approximately 40,000 and 45,000 daltons respectively. Both alpha and beta subunits have been cloned.[100] In its germline configuration, the gene for the beta subunit of the T-cell receptor exists as discontinuous gene segments: multiple variable regions (Vß) and two arrays of diversity (Dß1, Dß2), joining (Jß1, Jß2) and constant (Cß1, Cß2) regions (Figure 1).

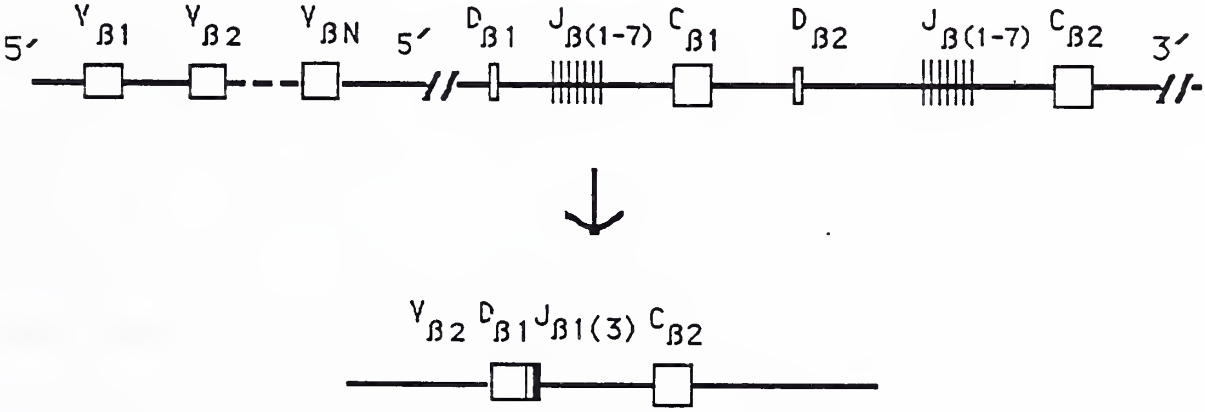

Figure 1. Rearrangement of the T-cell receptor ß chain genes.

As the T-cell matures the genes rearrange to generate a unique antigenic structure. First a D region gene segment rearranges to one of the J region segments. This DJ segment then combines with a V gene segment to form a VDJ assembly which is associated with either Cß1 or Cß2.[97] Inclusion of the D region in the final complex (V(D)JC) is optional.

Formation of a complete ß chain mRNA is accomplished by splicing. Somatic recombination results in thousands of possible combinations.

Millions of unique T-cell receptor antigen sites may be generated by the pairing of the rearranged alpha and beta subunits. In addition to somatic recombination, Ig genes undergo somatic mutation which contributes to the generation of antibody diversity.[101] This mechanism has not been demonstrated to contribute to the generation of diversity for T-cell receptors.

Molecular probes derived from the T-cell receptor ß chain genes can be used to identify distinctive clonal populations of T-cells. DNA from a chosen cell population is extracted, subjected to digestion with restriction endonucleases, size fractionated by agarose gel electrophoresis and then transferred to nitrocellulose paper according to the method of Southern.[102] Hybridization to the radioactively labelled probe is followed by autoradiography.

Each individual T-cell expresses a T-cell receptor with unique antigen specificity. Since all diploid cells contain two copies of every gene this means that there is expression of only one allele of a given antigen receptor gene in any cell. If the T-cell receptor loci on both chromosomes have undergone rearrangement, only one of these rearranged genes is functional, and only the functional rearrangement results in mRNA and protein expression. This phenomenon is referred

to as allelic exclusion. The specificity and efficiency of the immune system depends on this phenomenon. Thus, the ß chain of the T-cell receptor which is expressed on the cell surface results from translation of a specific mRNA sequence which has been transcribed from a single gene locus which has undergone rearrnagement.

When DNA extracted from peripheral blood containing normal polyclonal T-cells is analyzed by Southern blotting, distinct bands representing DNA in its germline or unrearranged configuration are seen. These germline bands reflect DNA from the chromosome which has not undergone rearrangement. DNA extracted from monocytes and granulocytes also contributes to the intensity of the germline bands.

If DNA extracted from a pure polyclonal T-cell population is analyzed, only the germline bands are seen. The unique rearrangements of the polyclonal T-cells result in thousands of restriction fragments which are represented as a faint smear. The persistence of the germline bands in a polyclonal T-cell population reflects those T-cells which have undergone a single rearrangement of the T-cell receptor. The T-cell receptor genes on the second chromosome remain in the germline configuration.

Rearrangement, which results in the deletion of intervening DNA, gives rise to a new restriction fragment by altering the 5′ restriction enzyme site. This new restriction fragment differs in size from the germline bands.

If DNA extracted from a cell population containing a monoclonal T-cell population is analyzed by this method, a new band, distinct from the germline bands is detected. This new band consists of DNA fragments which are identical in size as they are the result of the clonal expansion of a single cell; this results in a population of cells all of which carry the same rearrangement. (Figures 2,3) The monoclonal population must comprise at least 1% of the total population of cells in order for a new distinct band to be visible. The intensity of the new band is directly proportional to the percentage of cells which comprise the monoclonal population.

In some cases, a T-cell may undergo rearrangement of both chromosomes. However, only one of the rearrangements results in a functional T-cell receptor which is expressed on the cell surface. Nevertheless, the two distinct rearrangements are detectable by Southern blot analysis. In summary, a single monoclonal population may be reflected by the presence of a single new band, or by the presence of two new bands depending on whether one or both chromosomes have rearranged.

Numerous studies using this method to detect monoclonal populations of T-cells have been carried out on a wide range of lymphoproliferative disorders. Minden and Mak have summarized the spectrum of conditions which have been examined for rearrangements of the T-cell receptor ß chain

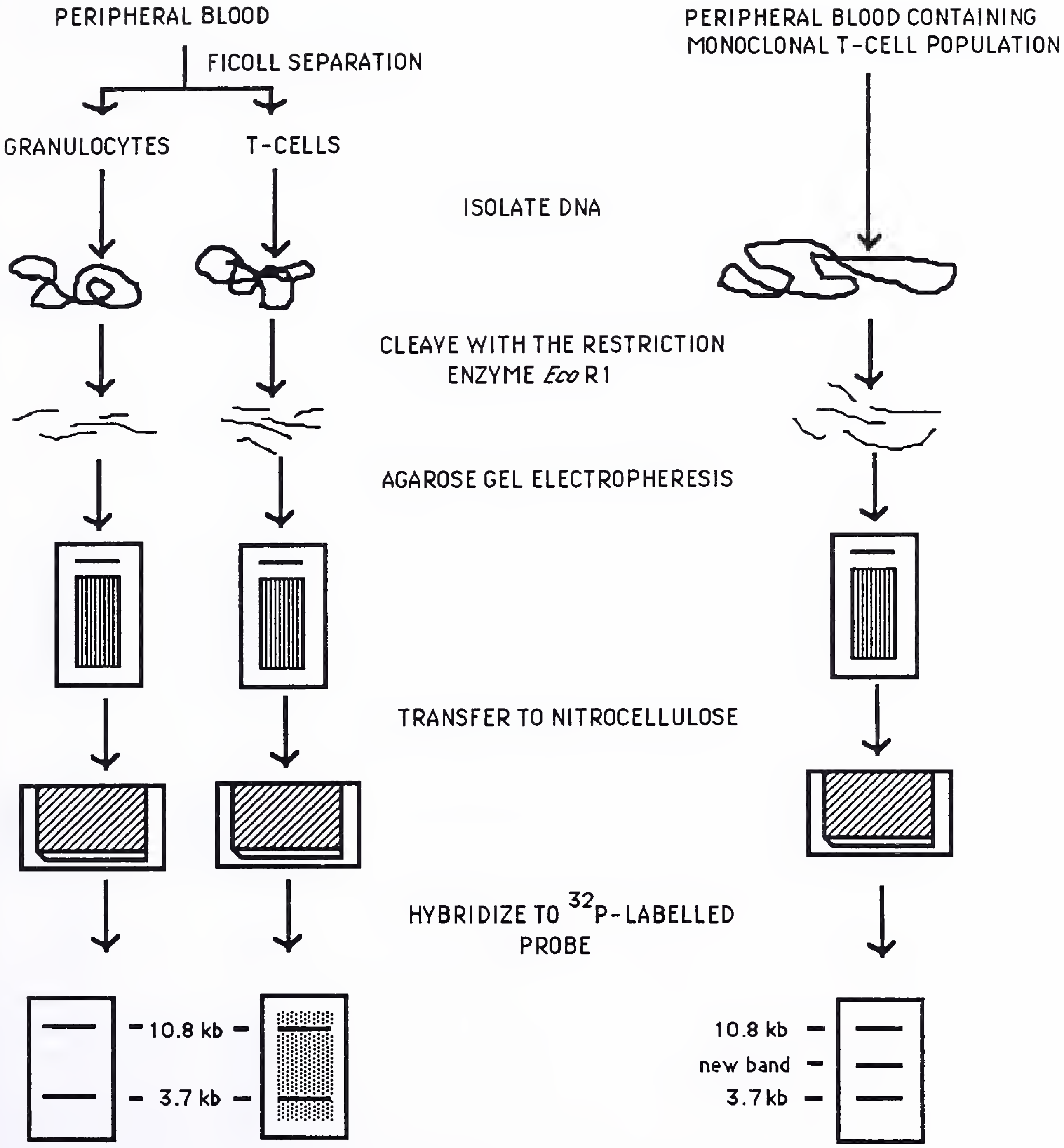

Figure 2. Southern blot analysis of the T-cell receptor ß chain genes following digestion with the restriction enzyme *Eco* R1.

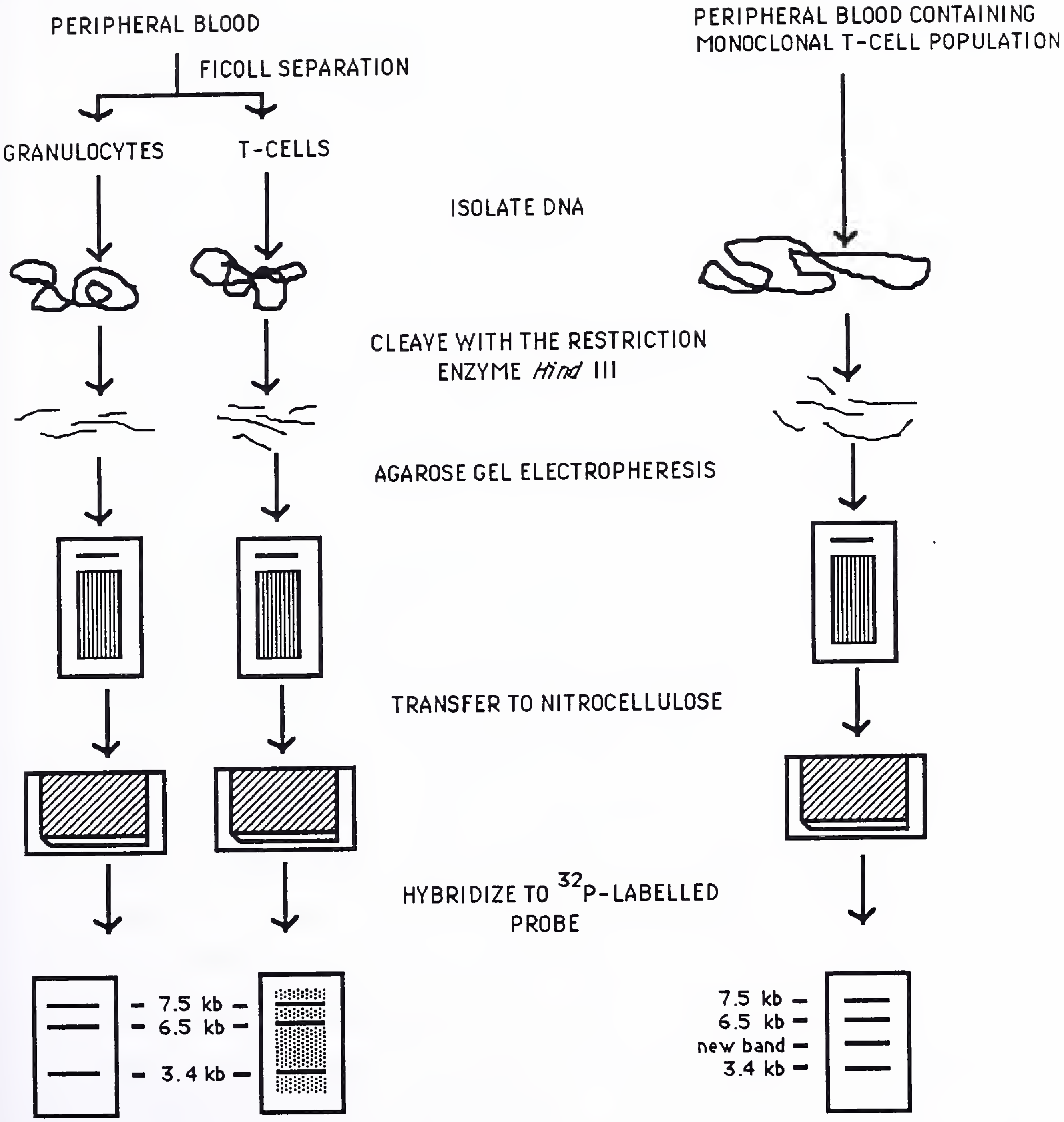

Figure 3. Southern blot analysis of the T-cell receptor ß chain genes following digestion with the restriction enzyme *Hind* III.

genes.[103] Several groups have shown that T-cell receptor ß chain gene rearrangements occur in patients with T-cell acute lymphoblastic leukemia (T-ALL), adult T-cell leukemia (ATL) and T-cell chronic lymphocytic leukemia (T-CLL). Unique rearrangements have been demonstrated in patients with HTLV-I associated adult T-cell lymphoma and nearly all non-Hodgkin's lymphomas with T-cell markers. Several groups including Berliner et al demonstrated unique T-cell receptor ß chain gene rearrangements in patients with T-cell lymphocytosis and cytopenia, a rare condition which is perhaps a form of T-cell CLL.[104]

Gene rearrangement studies are usually helpful in defining the lineage of neoplasms lacking lineage-specific surface determinants. However, not uncommonly, both Ig and T-cell receptor rearrangements are detectible in the same cell populations. Patients with non-B non-T ALL may have T-cell receptor ß chain rearrangements in addition to Ig heavy chain gene rearrangements.[105] Approximately 10% of patients with chronic B cell malignancies and B cell type non-Hodgkin's lymphoma have detectable T-cell receptor ß chain gene rearrangements.[13] This lineage infidelity[106] has been interpreted to mean that malignant transformation results in genetic deregulation. Alternatively, Greisser et al proposed that this dual pattern may reflect a clonal cell population that arose from an undifferentiatied hematopoietic cell capable of both Ig and T-cell receptor gene

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We proposed that this dual [illegible]

regulation took place from a [illegible]

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rearrangements prior to becoming committed to T or B cell lineage.[107] A minority of patients with AML have detectable rearrangements of the T-cell receptor ß chain genes.[108]

As with studies of Ig genes, the availability of probes for the T-cell receptor genes has provided a useful means of identifying monoclonal populations, establishing the lineage of ambiguous tumor populations, and diagnosing malignancy. In addition, this method is a sensitive means of detecting relapse at an early stage.[109]

As previously stated, T-cell receptor gene rearrangement studies have proven to be a sensitive and practical means of diagnosing CTCL and differentiating it from the benign dermatoses.[17] They also aid in the diagnosis of occult disease. In accordance with other data, Berger et al have demonstrated clonal rearrangements in 100% of patients with CTCL whom they studied. Four of the 13 patients with CTCL were also found to have immunoglobulin gene rearrangements.[110] Ralfkiaer et al employed genotypic analysis for improving the diagnosis of extracutaneous disease in advanced cases of CTCL.[57]

Plaque stage CTCL lesions from multiple sites in the same patient were examined in the same study, but the genes were found to be in their germline configuration. This suggests that T-cell proliferation at this stage may contain very small numbers (<1%) of monoclonal cells or may be

polyclonal. Most recently, Weiss et al proposed that T-cell receptor gene rearrangement studies provide the most sensitive means of detecting clonal malignant populations in the peripheral blood of patients with CTCL.[18]

In light of this background, we chose to use T-cell receptor ß chain gene rearrangement studies to test the hypothesis that BE2 is also a marker of T-cell clonality. For these studies, we have used BE2+ and BE2- populations of peripheral blood cells from patients with CTCL and unsorted peripheral blood cells from patients with PSS whose peripheral blood lymphocytes expressed high levels of BE2.

MATERIALS AND METHODS

Patient Samples: Peripheral blood cells were obtained from five patients with leukemic phase cutaneous T-cell lymphoma and from six patients with progressive systemic sclerosis. Blood was collected in heparinized tubes. Mononuclear cells were separated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. Five to 10 mls of whole blood were placed in a 50 ml conical tube and the volume was brought to 35 ml with Dulbecco's Ca^{++} and Mg^{++} free phosphate-buffered saline (PBS). Ten mls of Ficoll-Hypaque were layered onto the bottom of the tube and the tube was then centrifuged at 1500 rpm for 30 minutes. The mononuclear cells were removed from the interface, washed with 1X PBS and centrifuged at 1500 rpm for 10 minutes. The PBS was decanted, leaving a cell pellet.

Cell Marker Analysis: Determination of levels of BE2 surface antigen expression was performed by Peter Heald and Michael Girardi (Yale University School of Medicine). Aliquots of $5x10^5$ cells were incubated with saturating levels of BE2 (gift of Carole Berger, Ph.D.). Following primary incubation at 4 deg C for 30 minutes, tubes were centrifuged at 300 x g for 30 minutes and washed two times with Hanks Buffered Salt Solution (HBBS) with 10% AB serum

including .1% sodium azide. Cells then underwent a 20 minute incubation with goat anti-mouse FITC conjugate (Becton-Dickinson, Sunnyvale, CA). Cells were washed again and analyzed using a Cytofluorograf IIs/Cell Sorter (Ortho Diagnostic Systems, Raritan, NJ). Results were recorded as the percentage of positives over background.

Cell Sorting: Mononuclear cells separated from the peripheral blood of patients with CTCL were stained with the BE2 surface marker and then sorted using a Fluorescence-Activated Cell Sorter (Becton-Dickinson, Sunnyvale, CA) into BE2+ and BE2- populations by Rocco Carbone (Yale University School of Medicine).

DNA Isolation: High molecular weight genomic DNA was prepared as follows. Mononuclear cells were incubated overnight at 37 deg C in 10 mM Tris/.1 M NaCL/.01 M EDTA/.5% sodium dodecyl sulfate with 200 ug/ml of Proteinase K. The DNA was then extracted once with phenol/chloroform/isoamyl alcohol and several times with chloroform/isoamyl alcohol. Following extraction, DNA was precipitated with ethanol and 10% volume 3M sodium acetate and resuspended in 10 mM Tris/1 mM EDTA, pH 7.5 (TE).[111] The concentration of DNA was determined by optical density measurement at A_{260} using a spectrophotometer.

Southern Blot Analysis: Southern blot analysis was performed on genomic DNA from each patient including DNA isolated from both BE2+ and BE2- mononuclear cell populations from patients with CTCL. Genomic DNA (12 ug/sample) was digested at 37 deg C with the restriction enzymes *Eco* R1 and *Hind* III (New England Biolabs, Beverly, MA). Several samples were also digested with *Bam* H1 (New England Biolabs, Beverly, MA). Digested DNA was then size fractionated on .9% agarose gel and photographed. The gels were denatured in 1.5 M sodium chloride/.5 M sodium hydroxide, neutralized in 1 M Tris (pH 8.0)/1.5 M sodium chloride, and the DNA was transferred to nitrocellulose paper according to the method of Southern.[101] Filters were hybridized overnight at 42 deg C in 40% formamide/4X SSC (.6 M sodium chloride/.06 M sodium citrate)/.01 M Tris/1X Denhart's solution/ 10% dextran sulfate, containing salmon sperm DNA (100 ug/ml) and radioactive probe ($1x10^6$ cpm/ml). Following hybridization, filters were washed with 2X SSC (.3 M sodium chloride/.03 M sodium citrate)/.1% SDS for 20 minutes at room temperature and for 20 minutes at 55 deg C and then in .1X SSC (.15 M sodium chloride/.0015 M sodium citrate) /.1% SDS for 40 minutes at 55 deg C. Filters were autoradiographed for 12-18 hours at -70 deg C with an intensifying screen.

Probe: A *Bgl* II-*Bgl* II probe containing the Cß2

region of the T-cell receptor gene was prepared by enzymatic digestion, with the restriction endonucleases *Eco* R1 and *Bgl* II, of a propagated *Escherichia coli* plasmid containing the T-cell receptor ß chain gene (gift from J.G. Seidman). The digested plasmid DNA was then run on a 1% agarose gel. A slice of the gel containing the 1 kb fragment corresponding to the Cß2 region of the T-cell receptor gene was cut out and the DNA recovered from the gel by electroelution (Elutrap: Schleicher & Schuell, Keene, NH). Concentration of the isolated probe was determined by analysis on agarose gel.

The purified probe was radiolabelled with ^{32}P by nick translation according to the method of Rigby et al.[110] Briefly, .2 ug of DNA was dissolved in distilled water and incubated at 16 deg C for 35 minutes with the nucleotides dATP, dGTP, dTTP at a concentration of .4 mM/L, ^{32}P labelled dCTP (Amersham, Arlington Heights, IL), 10X buffer and an enzyme mixture containing DNA polymerase I and DNase I in 50% (v/v) glycerol (Nick Translation kit, Boehringer Mannheim Biochemicals, Indianapolis, IN). The reaction was then stopped with .5 M EDTA/1X STE (10 mM Tris/100 mM NaCl/1 mM EDTA) and passed over a G50 Sephadex Column (Boehringer Mannheim). One ul was placed on a DE 81 filter and washed successively with .5 M Na_2HPO_4/H_2O/Ethanol. Three mls of scintillation fluid was added to a small vial containing the DE 81 filter and the number of cpm in one ul of sample was

determined in a liquid scintillation counter.

RESULTS

CLINICAL PROFILES: **CTCL Patients.** Each patient had advanced stage CTCL with leukemic involvement. The clinical characteristics of these patients are outlined in Table 1.

Table 1. Clinical Characteristics of Patients with CTCL

CASE	SEX	AGE	WBC	SÉZARY CELLS	PRIOR TREATMENT	RESPONSE
51	M	55	9.3	10%	None	+
68	M	61	15.7	6%	Acutane, Tanning booth, MTX	+
55	M	42	22.2	19%	PUVA, topical mustard	+
85	F	72	14.1	4%	Prednisone, UVB, topical steroids, N2M	+
70	M	52	11.8	7%	topical steroids, total body electron-beam, Adriamycin Cytoxan, PUVA	+

WBC, white blood count (k/mm^3); RESPONSE, response to photopheresis.

PSS Patients: Each patient had a positive diagnosis of PSS by skin biopsy for less than 2 years and had 30% or greater increase in skin involvement in the last six months. The patients are all part of a randomized clinical trial of D-penicillamine versus photopheresis for treatment of their disease. I am the blinded observer for this trial which is currently still in progress. Therefore clinical data on these patients are not yet available to me.

IMMUNOPHENOTYPIC ANALYSIS: Results of BE2 surface antigen expression are summarized in Table 2. No data were available for PSS patient #4. BE2 expression for PSS patient #2 was recorded as divided percentages of lymphocytes and monocytes expressing BE2.

Table 2. BE2 Surface Antigen Expression on Peripheral Blood Cells

CASE	DIAGNOSIS	% BE2+ OVER BACKGROUND
51	CTCL	57%
68	CTCL	70%
85	CTCL	54%
55	CTCL	34%
70	CTCL	17%
1	PSS	25%
2	PSS	10% lymphs 72% monos
3	PSS	19%
4	PSS	ND
5	PSS	24%
6	PSS	36%

ND, not determined.

CELL SORTING: CTCL cases 68, 85 and 55 were sorted by FACS. In case 68 the BE2+ population was the high 35% of the sort. The BE2- population was the low 10% of the sort. In case 85 the BE2+ population was the high 30% of the sort, and the BE2- population the low 30% of the sort. In case 55 the BE2+ population was the high 15% of the sort, and the BE2- population was the low 20% of the sort. Cases 51 and

70 were sorted using magnetic beads into BE2+ and BE2- populations.

DETECTION OF T-CELL RECEPTOR ß CHAIN REARRANGEMENT: The germline organization of the ß chain locus is diagrammed in Figure 4.

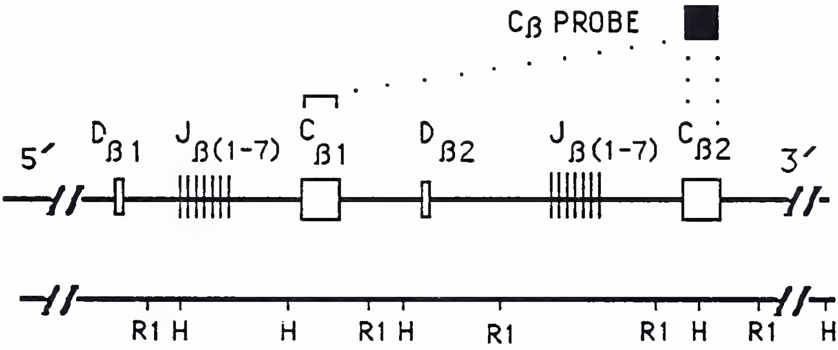

Figure 4. Germline organization of the T-cell receptor ß chain genes. The locations of the *Eco* R1 and *Hind* III restriction-endonuclease sites are indicated. Southern blot analysis was performed using a 1 kb *Bgl* II - *Bgl* II probe from Cß2 which recognizes both constant regions.

genes. The Cß genes are present on two *Eco* R1 fragments of 10.8 kb and 3.7 kb (Figure 2). The 10.8 kb fragment is the result of hybridization to Cß1. This fragment contains the Jß1 gene region, allowing rearrangement into this region to be detected. The 3.7 kb fragment results from hybridization to Cß2. This fragment does not contain a J region thereby

making rearrangement in this region undetectable by this digest. The Cß genes are present on three *Hind* III fragments of 7.5 kb, 6.5 kb and 3.4 kb (Figure 3). The 7.5 kb band results from hybridization to a Cß2 fragment containing the Jß2 region. Thus rearrangements involving Cß2 will be detected due to an alteration in the 5′ restriction site. The 6.5 kb and 3.4 kb fragments result from hybridization to Cß2 and Cß1, respectively. These fragments lack J regions, and therefore do not allow detection of rearrangements. Thus digestion with *Eco* R1 allows detection of T-cell receptor ß chain rearrangements involving Cß1, and digests using *Hind* III allow detection of Cß2 gene rearrangements.

Unique T-cell receptor ß chain rearrangements were detected in unsorted samples from all CTCL patients studied. Furthermore, the rearrangements detected were present in both BE2+ and BE2- populations. Four of these cases are illustrated in Figure 5. Case 51 showed rearrangement of Cß1 and Cß2. All other cases revealed only rearrangement of Cß2.

The rearranged band in Case 51, indicated by an arrow, occurs at approximately 7 kb. The germline bands are present at 10.8 kb and 3.7 kb. In the unsorted population, an extra band is present at 9 kb. This band suggests rearrangement of Cß1, but has been noted in DNA extracted from the peripheral blood of normal controls.[103] This artifact has also been detected in *Eco* R1 digests of DNA extracted from fibroblasts.[14] Figure 6 illustrates the presence of this

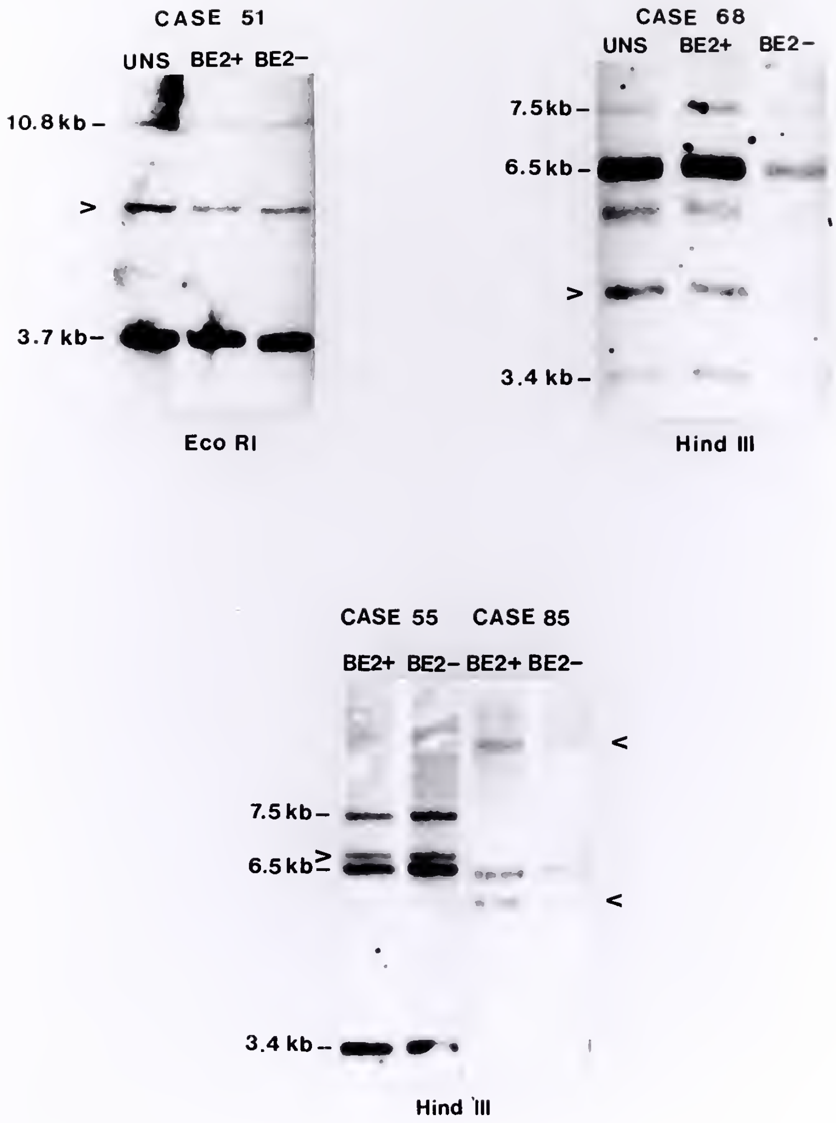

Figure 5. Southern blot analysis of DNAs from patients with CTCL. *Eco* R1 and *Hind* III digested DNAs were size-fractionated on .9% agarose gels and blotted on nitrocellulose as described in the Materials and Methods. Filters were hybridized to the Cß2 probe. Autoradiograms revealed extra bands indicative of somatic rearrangements in all 4 cases. Rearranged bands are indicated by arrows. In all cases, identical banding patterns were observed in unsorted (UNS), BE2+ and BE2− cell populations. Case 51 revealed rearrangement of Cß1. An aberrant band representing an artifact is present in the unsorted population. The extra bands in cases 68, 55 and 85 indicate rearrangement of Cß2. The extra band seen in all populations in case 68 is most likely the result of contaminating genomic sequences in the probe.

Figure 1 [illegible] with CTL [illegible] DNA [illegible] alkaline-transfer [illegible] nitrocellulose [illegible] filters were hybridized [illegible] revealed extra bands indicating [illegible] all 4 cases. Rearranged bands [illegible] all cases, identical banding pattern [illegible] unsorted (UNS), NK1+ and NK1- cell populations [illegible] revealed rearrangement of [illegible]. An aberrant band [illegible] an artifact is present in the unsorted population. The extra bands in cases 16, 55 and 80 indicate rearrangement of the [illegible] The extra band seen in all populations in case 67 is most likely the result of cross-hybridizing genomic sequences in the probe.

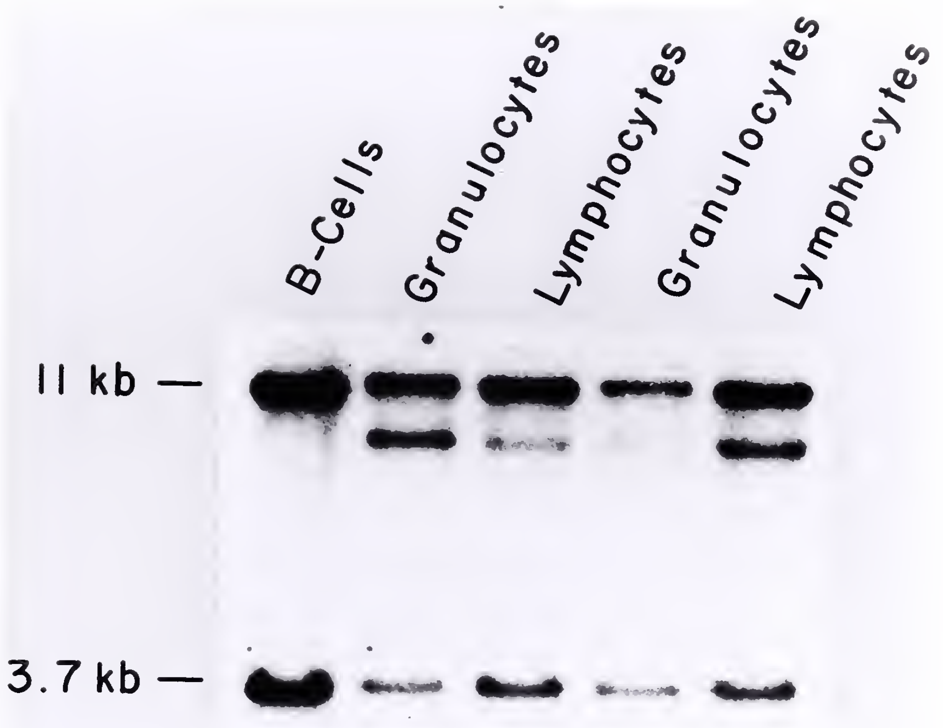

Figure 6. Autoradiogram demonstrating the presence of an aberrant band in *Eco* R1 digests of granulocytes and lymphocytes from normal controls. This artifact is probably of no biologic significance.

band in granulocytes and lymphocytes from 2 normal individuals. This aberrant band is not visible in the BE2+ and BE2- sorted cell populations from Case 51. This finding most likely reflects the low DNA yield obtained from these sorted specimens. Even the germline bands are less intense in these populations reflecting the fact that fewer micrograms of DNA were available for Southern blotting. The decrease in the intensity of the 10.8 kb band relative to the other bands is explained by the presence of the monoclonal population which has rearranged to the Cß1 locus on one chromosome.

In addition, digestion with the restriction enzyme *Hind* III revealed the presence of an extra band representing a second rearrangement involving Cß2. Rearrangement to Cß2 necessitates the deletion of the Cß1 locus; this results in a further decrease in the intensity of the 10.8 kb band which reflects hybridization to Cß1. Thus the monoclonal T-cell population in case 51 has rearranged on both chromosomes: to Cß1 on one chromosome and to Cß2 on the other. This results in the presence of an extra band in both *Eco* R1 and *Hind* III digests. The continued presence of the 10.8 kb germline band indicates that normal mononuclear cells remain. The monoclonal population does not comprise 100% of the total T-cell population. The germline band is present in both BE2+ and BE2- populations demonstrating unequivocally the presence of BE2+ normal cells in a patient with CTCL.

In Case 68 a unique rearrangement involving Cß2 results in a distinct band indicated by an arrow in Figure 5. The germline bands at 7.5 kb and 3.4 kb are decreased in intensity. The 7.5 kb fragment, which contains the J region of the Cß2 locus, is appropriately reduced in intensity as this locus is utilized in the new rearrangement. A decrease in the 3.4 kb band which results from hybridization to Cß1, also reflects the rearrangement to Cß2 which deletes Cß1. An extra band is seen below the 6.5 kb band. This band was present in all lanes probed including lanes containing DNA extracted from patients without a detectable clonal population. In addition, the extra band is relative to DNA concentration. It is most likely the result of contaminating genomic sequences in the probe. Additional DNA was not available to repeat the blot. The relative faintness of the bands in the BE2- population is secondary to the low DNA yield obtained from these cells. Nevertheless, it is clear that no difference exists in the banding patterns seen in the three cell populations.

Cases 55 and 85 were run in parallel. The three germline bands are shown clearly in Case 55. A new band representing a unique rearrangement of Cß2 is seen just above the 6.5 kb germline band. This band is decreased in intensity when compared to the 6.5 kb and 3.4 kb germline bands. The 7.5 kb band which contains the J region of Cß2 is appropriately decreased in intensity in accordance with the [illegible] is a [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] that [illegible] band [illegible] yield [illegible] that no differen[illegible] three cell populations.

Cases 15 and 16 were [illegible] gasoline units are shown. Lane [illegible] representing a unique new [illegible] the 6.5 kb gasoline band. [illegible] intensity when compared to the [illegible] bands. The 7.5 kb band which comprises the 3' region of [illegible] appropriately decreased in intensity in accordance with [illegible] presence of a clonal population which rearranges to Cß2.

Case 85 is particularly interesting. The 6.5 kb germline band is visible. This band, which results from hybridization to a Cß2 containing fragment which hybridizes to the 3´ end of the probe is unaffected by rearrangement into either Cß1 or Cß2. Because the 5´ end of the hybridizing fragment does not include the J region, it is invariant. However, the other two germline bands have been deleted. Two new bands, indicated by arrows, are present in both BE2+ and BE2- populations. These bands reflect a monoclonal T-cell expansion. These bands correspond to two rearrangements of Cß2, reflecting a population in which the T-cell ß chain locus has rearranged on both chromosomes. The absence of the two germline bands indicates that 100% of the cells are tumor cells. No normal cells containing germline DNA remain.

The 3.4 kb fragment, which reflects hybridization to Cß1, is not visible because the two rearrangements to the Cß2 locus result in deletion of Cß1 on both chromosomes. The 7.5 kb fragment containing the J region of Cß2 has also completely disappeared as all of the cells have undergone rearrangement which necessitates an alteration in the restriction site pattern. Both BE2+ and BE2- populations demonstrate identical banding patterns. Furthermore, the observation that 100% of the cells belong to a single clonal expansion provides definitive evidence that both BE2+ and

BE2- tumor cells exist.

Rearrangements involving the T-cell receptor ß chain were not detected by Southern blotting in any of the DNA samples extracted from patients with PSS. DNA was in the germline configuration in all samples. Six of these cases are illustrated in Figure 7. The above mentioned *Eco* R1 artifact was observed in Cases 3 and 5. A decrease in the intensity of the 10.8 kb fragment is particularly striking in cases 2 and 4. This finding indicates the presence of a significant polyclonal T-cell population in these peripheral blood samples.

As explained above, this 10.8 kb fragment results from hybridization to a Cß1 gene segment which contains a J region. This fragment is altered in size by rearrangements using Cß1. The Cß1 locus is deleted by rearrangements to Cß2. Therefore, a decrease in the intensity of the 10.8 kb band occurs when a large polyclonal T-cell population is present.

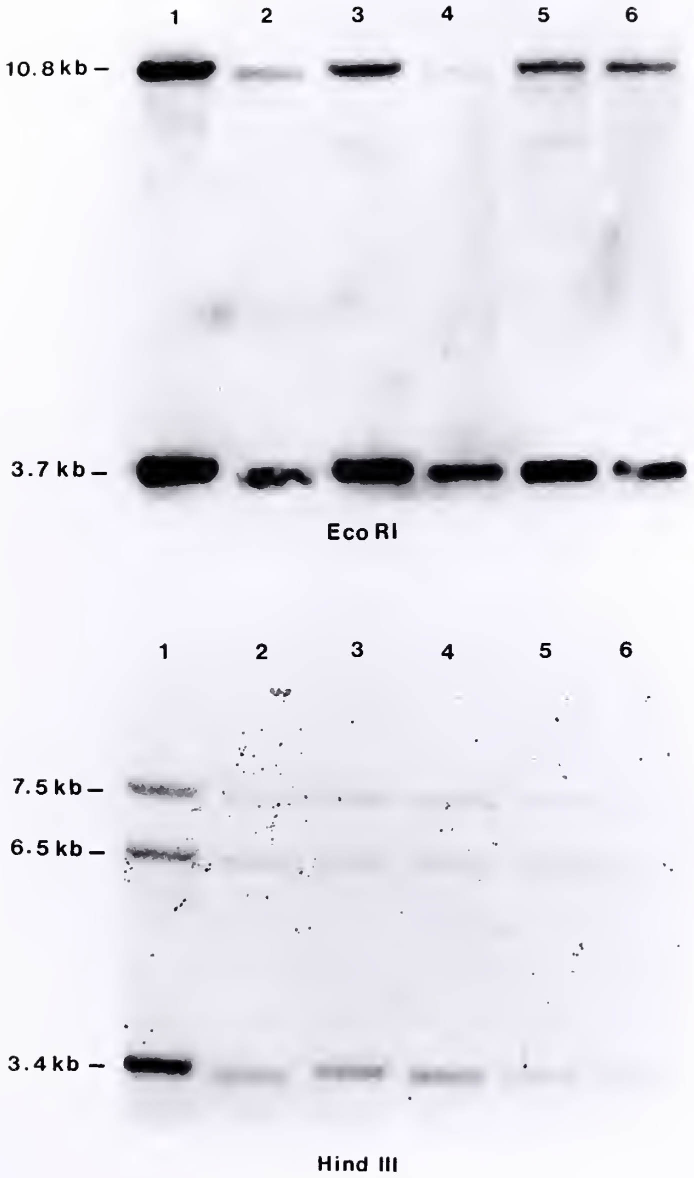

Figure 7. Autoradiograms of *Eco* R1 and *Hind* III digests of DNAs from 6 patients with PSS. In each case, DNA is present in its germline configuration. The *Eco* R1 artifact is visible in cases 3 and 5. The decreased intensity of the 10.8 kb band relative to the 3.7 kb band in cases 2 and 4 reflects the presence of a large polyclonal T-cell population.

DISCUSSION

The detection of clonal T-cell populations by DNA analysis has contributed greatly to our understanding of many lymphoproliferative disorders. This technology has also improved our ability to diagnose clonal disorders and to detect disease recurrence. In this study, we have applied gene rearrangement studies, using the ß chain of the T-cell receptor, to investigate the origins of cells bearing the BE2 surface marker. We have shown that surface expression of BE2 does not predict a clonal T-cell expansion in either CTCL or PSS.

Peripheral blood cells from patients with CTCL were sorted into BE2- and BE2+ populations by FACS. As previously stated, Southern blot analysis of the T-cell receptor ß chain genes is capable of detecting a clonal population which comprises as little as 1% of the total population of cells.[14,16] Although the cells were meticulously sorted, the possibility of some overlap between the two populations of cells exists. However, identical banding patterns were observed in both populations. There was no detectable difference in the percentage of monoclonals in the BE2+ and the BE2- populations.

The finding that 100% of the cells containing DNA in case 85 are tumor cells renders our conclusion that both BE2+ and BE2- clonal tumor cells exist in CTCL unambiguous.

Since the cells are not 100% BE2+, BE2 does not identify the monoclonal T-cell population in CTCL. In addition, case 51 definitively demonstrates the presence of BE2+ normal mononuclear cells. Consequently, we have detected both BE2+ normal cells and BE2- tumor cells in the peripheral blood of patients with CTCL. Additional evidence that BE2 does not correlate with T-cell clonality is provided by our data on PSS patients.

The finding of an aberrant band in *Eco* R1 digests from the unsorted cell population from Case 51 and from normal individuals illustrated in Figure 6 deserves mention. This artifact, which as noted by other investigators does not correspond to a clonal T-cell expansion,[103,12] is probably of no biological significance. However, this technical aberrancy necessitates that rearrangements of Cß1 be interpreted cautiously.

There are several important clinical conclusions which may be drawn from our study. To begin with, BE2 expression does not correlate with tumor burden in CTCL. While BE2 may be a cell surface marker expressed on lymphocytes in the peripheral blood of approximately 75% of patients with CTCL, both BE2+ and BE2- tumor cells and BE2+ normal mononuclear cells are present in the peripheral blood of patients with CTCL. This has several implications.

First, a tumor specific marker can be tagged with a number of toxic compounds which could selectively eliminate a

tumor population. It is clear from these studies that BE2 will not be useful as a new weapon in our therapeutic armamentarium.

Second, if BE2 were a tumor specific marker, it could potentially be a useful diagnostic tool in CTCL, especially since surface marker analysis is now routinely performed at many medical centers. In particular, it would be useful for detecting early peripheral blood involvement. Surface marker analysis is both more rapid and less labor intensive than gene rearrangement studies. Our results militate against the diagnostic usefulness of BE2 in CTCL.

The clinical case histories of the patients studied in this thesis underscore the frequently noted diagnostic challenge CTCL poses in its early stages. In case 51, the patient was initially thought to have a fungal skin infection. Over a year elapsed during which the patient in case 68 had prominent cutaneous manifestations until the diagnosis of CTCL was made. The patient in case 85 was treated with prednisone for an elusive "inflammation" before her diagnosis was made. Similarly, in case 70 the patient was treated with moisturizers and topical steroids for a waxing and waning eruption for two years prior to his diagnosis.

The usefulness of gene rearrangement studies in the detection of leukemic progression in CTCL has not been extended to the diagnosis of plaque or early stage CTCL.

Early diagnosis has well-documented therapeutic importance. Ralfkiaer et al analyzed DNA extracted from multiple skin biopsy specimens from 8 patients with plaques clinically suspicious, but histologically nondiagnostic, for early CTCL and 4 patients with plaque or stage II mycosis fungoides.[57] In each case, germline configuration of the T-cell receptor ß chain gene was demonstrated.

In contrast, DNA from skin biopsy samples from patients with advanced CTCL revealed T-cell receptor ß chain rearrangement. The author concludes that this method is not a useful diagnostic test for early CTCL, perhaps because the disease is polyclonal at this stage or perhaps because a monoclonal expansion if present is below the level of detection of this method. It would be interesting to know if those patients in whom rearrangements were not detected went on to develop advanced CTCL because only 4 of 12 had histologic evidence of CTCL. Additional studies to evaluate the usefulness of this technique in early CTCL should be done.

In a paper proposing BE2 as an activation marker, Heald and Berger stated that future studies should aim to identify biologic differences between BE2+ and BE2- cell populations.[5] Our findings raise the possibility that CTCL is a dynamic disease in which tumor cells may convert from a BE2- to a BE2+ state or vice versa. The in vitro induction of BE2 expression on BE2- cells from CTCL patients has been shown.

We have further demonstrated the simultaneous in vivo existence of BE2+ and BE2- tumor cells and BE2+ normal cells. This data concurs with the idea that BE2 is an activation marker.

If BE2 is an activation marker whose expression can be induced on normal lymphocytes in vitro, and if it does not define the monoclonal population in CTCL, then what is its role in the response to photopheresis? In the initial trial of photopheresis, decreased BE2 expression was correlated with clinical response in a subgroup of patients who responded to the treatment. Our study indicates that although alterations in BE2 expression may be an empiric marker of clinical response to photopheresis, they probably do not correlate directly with cytotoxic activity against the malignant clone. This conclusion is further supported by the observation that in our group of 5 patients with advanced CTCL, BE2 expression remained relatively constant in 2 patients, decreased in one and became elevated in another patient who also showed a dramatic increase in his white count. A fifth patient who was markedly positive at the time of our study, had been previously BE2- and converted to BE2 positivity after beginning photopheresis treatment. Despite this heterogeneity of response of BE2 expression, all of these patients showed response to photopheresis.

It has been proposed that scleroderma may be a T-cell mediated disease.[8] However, there are no reports in the

literature of studies attempting to identify a clonal T-cell expansion in the peripheral blood of patients with PSS. We hypothesized that BE2 expression may identify a monoclonal T-cell expansion in PSS. To test this hypothesis, we analyzed DNA extracted from mononuclear cell preparations from patients with PSS for evidence of a monoclonal T-cell population. Germline configurations of the ß chain genes were found in each case, confirming that BE2 expression does not correspond to a monoclonal T-cell expansion in the peripheral blood of patients with PSS. Furthermore, our studies confirm that if a clonal T-cell expansion is present in the peripheral blood of patients with PSS it comprises less than 1% of the total peripheral blood cell population. It is conceivable that a pathogenic T-cell clone may reside in the skin or other affected organs of patients with PSS. Cellular epidermal infiltrates seen in patients with PSS are comprised primarily of T-cells.[8] Gene rearrangement studies on DNA extracted from skin biopsy specimens could be done to determine if these T-cells are the result of a monoclonal expansion.

The lack of an identifiable monoclonal T-cell expansion in the peripheral blood of patients with PSS has other implications. First, if photopheresis induces a clonotypic response, then one would expect its efficacy in PSS to be limited. The results of this trial are not complete; however, if photopheresis does prove to be effective in PSS,

then this theory becomes less plausible as a therapeutic mechanism in PSS. Instead, a more general mechanism of action would be likely.

The sustained clinical response seen in CTCL patients successfully treated with photopheresis suggests some sort of immunologic suppression of the malignant clone. Surely, the responses achieved with photopheresis are out of proportion to simple cytotoxic destruction of malignant cells in the peripheral blood. Results from studies employing animal models have suggested that photopheresis may induce specific suppression of immunoactive T-cells.[82] Generalized suppression of activated polyclonal T-cells which would be capable of B cell stimulation could conceivably have an effect on the progression of autoimmune disease. This hypothesis could be investigated by comparing the interaction of serum and tumor cells extracted from a CTCL patient before and after photopheresis. This might indicate if the serum contains factors which are cytotoxic to the tumor cells. A cellular mechanism could be investigated by similar studies aimed at looking at the interaction of patients' T and B cells pre- and post therapy.

The presence of BE2 in CTCL and PSS, both systemic diseases with cutaneous manifestations, is intriguing. If BE2 is an activation marker, it would be useful to know if it is expressed in other diseases with cutaneous manifestations such as chronically transfused patients, ARC patients who

present with eczema, or patients with drug rashes secondary to phenytoin or methyldopa. In addition, it would be important to determine if it is expressed on the peripheral blood cells of patients with other purportedly T-cell mediated diseases for which trials of photopheresis are underway, particularly if BE2 expression may help predict which patients will respond to photopheresis.

In vivo activated T-cells have been demonstrated in the peripheral blood and CSF of patients with multiple sclerosis[112], and very late activation antigens have been detected on synovial fluid lymphocytes from patients with rheumatoid arthritis.[113] Additional investigative studies should help to define better the role of activated T-cells in autoimmune disease.

Southern blot analysis of the T-cell receptor genes is now being used to help understand the pathogenesis of a variety of autoimmune diseases. Distinct rearrangements of the T-cell receptor ß chain have been demonstrated in cultured T-cells from RA synovial infiltrates.[114] T-cells extracted from the peripheral blood of RA patients showed no evidence of rearrangement. Synovial fluid mononuclear cells also showed no evidence of ß chain rearrangement.[115] Tesch et al recently applied this technique to the analysis of the thymus in myasthenia gravis.[116] Gene rearrangement studies contributed greatly to our understanding of lymphoproliferative disorders. Their application to the

study of autoimmune disease may contribute to our understanding of its pathogenesis.

Our study may be viewed as an important step in further characterizing the BE2 surface antigen and further understanding its significance and usefulness with respect to photopheresis. The technology needed to clone BE2 is now available. The cloning of BE2 would allow investigators to compare the antigen to known sequences and to design experiments to elucidate its function. In the meantime, additional clinical studies such as those proposed above could establish the marker as an important diagnostic tool. Finally, understanding BE2 may help to elucidate the mechanism of photopheresis - a powerful therapeutic tool which may become even more useful once its mechanism of action is understood.

REFERENCES

1. Patterson, J.A.K., Edelson, R.L. Cutaneous T-Cell Lymphoma and Other Leukemic and Lymphomatous Infiltrates of the Skin in **Dermatology in General Medicine** 3rd Ed. Edited by T.B. Fitzpatrick, A.Z. Eisen, K. Wolff, I.M. Freedberg and K.F. Austen. New York, McGraw-Hill, 1987, pp. 1086-1118.

2. Berger, C., Morrison, S., Chu, A., Patterson, J., Estabrook, A., Takezaki, S., Sharon, J., Warburton, D., Iriguyen, O., Edelson, R. Diagnosis of Cutaneous T-Cell Lymophoma by use of Monoclonal Antibodies Reactive with Tumor Associated Antigen. **J Clin Invest** 70: 1205-1215, 1982.

3. Edelson, R.L., Berger, C., Gasparro, F., Jegasothy, B., Heald, P., Wintroub, B., Vonderheid, E., Knobler, R., Wolff, K., Palewig, G., McKiernan, G., Christiansen, I., Oster, M., Honigsmann, H., Wilford, H., Kokoshica, E., Rehle, T., Perez, M., Stingl, G., Laroche, L. Treatment of Cutaneous T-Cell Lymphoma by Extracorporeal Photochemotherapy. **N Eng J Med** 316: 297-303, 1987.

4. Edelson, R. L. Update on Photopheresis. **Yale J Biol Med** (in press).

5. Heald, P.W., Berger, C., Yamamura, T., McNamara, J., Edelson, R. BE-2 Antigen: Appearance in Activation and Long Term Growth of T-cells **J Invest Derm** (in press).

6. Berger, C.L., Friedman-Lien, A.E., Difranco, M., Rehle, T., Ostreicher, R., Knobler, R., Donofrio, S., Laubenstein, L.J., Edelson, R.L. Tumor-Associated Antigen is Expressed on Lymphocytes from Patients with Acquired Immune Deficiency Syndrome. **J Inves Derm** 87: 280-283, 1986.

7. Heald, P., Girardi, M., Kelleher, M., Edelson, R. Characterization of Peripheral Blood Lymphocytes in Patients with Progressive Systemic Sclerosis on the Basis of Activation Antigen Expression (in preparation).

8. Roumm, A.D., Whiteside, T.L., Medsger, T.A., Rodnan, G.P. Lymphocytes in the Skin of Patients with Progressive Systemic Sclerosis. **Arth Rheum** 27: 645-653, 1984.

9. Yanigi, Y., Yoshikai, Y., Legett, K, Clark, S.P., Aleksander, I., Mak, T.W. A Human T Cell Specific cDNA Clone Encodes a Protein Having Extensive Homology to Immunoglobulin Chains **Nature** 308: 145-149, 1984.

10. O'Connor, W.J.J., Wainscoat, J.S., Weatherall, D.J., Gatter, Pallesen, G., Ramsey, A., Stein, H., Wright, D.H., Mason, D.Y. Rearrangement of the T-cell Receptor ß-Chain Gene in the Diagnosis of Lymphoproliferative Disorders. **Lancet** 1: 1295-1299, 1985.

11. Bertness, V., Kirsch, I., Hollis, G., Johnson, B., Bunn, P.A. T Cell Gene Rearrangements as Clinical Markers of Human T Cell Lymphomas. **N Eng J Med** 313: 534-538, 1985.

12. Aisenberg, A.C., Kruntiris, T.G., Mak, T.W., Wilkes, B.M. Rearrangement of the gene for the Beta Chain of the T-cell Receptor in T-cell Chronic Lymphocytic Leukemia and Related Disorders. **N Eng J Med** 313: 529-533, 1985.

13. Waldmann, T.A., Davis, M.M., Bongiovanni, K.F., Korsemeyer, S.J. Rearangements of Genes for the Antigen Receptor on T Cells as Markers of Lineage and Clonality in Human Lymphoid Neoplasms. **N Eng J Med** 313: 776-783, 1985.

14. Minden, M.D., Toyonaga, B., Ha, K., Yanagi, Y., Chin, B., Gelfand, E., Mak, T. Somatic Rearrangement of T-cell Antigen Receptor Gene in Human T-Cell Malignancies. **Proc Natl Acad Sci** 82: 1224-1227, 1985.

15. Flug, F., Pelicci, P-G., Bonetti, F., Knowles, D.M., Dalla-Favera, R. T-Cell Receptor Gene Rearrangements as Markers of Lineage and Clonality in T-cell Neoplasms. **Proc Natl Acad Sci** 82: 3460-3464, 1985.

16. Minden, M.D, Mak, T. The Structure of the T-Cell Antigen Receptor Genes in Normal and Malignant T-Cells. **Blood** 68: 327-336, 1986.

17. Weiss, L.M., Hu, E., Wood, G.S., Moulds, C., Cleary, M.L., Warnke, R., Sklar, J. Clonal Rearrangements of T-cell Receptor Genes in Mycosis Fungoides and Dermatopathic Lymphadenopahy. **N Eng J Med** 313: 539-544, 1985.

18. Weiss, L.M., Wood, C.S., Hu, E., Abel, E.A., Hoppe, R.T., Sklar, J. Detection of Clonal T-Cell Receptor Gene Rearrangements in the Peripheral Blood of Patients with Mycosis Fungoides/Sézary Syndrome. **J Invest**

Derm 92: 601-604, 1989.

19. Edelson, R.L. Cutaneous T-Cell lymphoma. **J Dermatol Surg Oncol** 6: 358-368, 1980.

20. Broder, S., Bunn, P.A. Jr. Cutaneous T-Cell Lymphomas. **Semin Oncol** 7: 310-331, 1980.

21. Edelson, R.L. Cutaneous T-Cell Lymphoma: Mycosis Fungoides, Sézary Syndrome and other Variants. **J Am Acad Derm** 2: 89-106, 1980.

22. Edelson, R.L., Fink, J.M. The Immunologic Function of Skin. **Sci Am** 252: 46-53, 1985.

23. Edelson, R.L., Kirkpatrick, C.H., Shevach, E.M., Schein, P.S., Smith, P.S., Smith, R.W., Green, I., Lutzner, M. Preferential Cutaneous Infiltration by Neoplastic Thymus-Derived Lymophocytes. Morphologic and Functional Studies. **Ann Intern Med** 80: 685-692, 1974.

24. Berger, C.L., Warburton, D., Raafat, J., LoGerfo, P., Edelson, R.L. Cutaneous T-Cell Lymphoma: Neoplasm of T-cells with Helper Activity. **Blood** 53: 642-651, 1974.

25 Broder, S., Edelson, R.L., Lutzner, M.A., Nelson, D.I., MacDermott, R.P., Durm, M.E., Goldman, C.K., Meade, B.D., Waldmann, T.A. The Sézary Syndrome: A Malignant Proliferation of Helper T Cells. **J Clin Invest** 58: 1297-1306, 1976.

26. Edelson, R.L. Cutaneous T-Cell Lymphoma in **Dermatology Update.** Edited by S. Moschella. New York, Elsevier Press, 1979, pp. 195-207.

27. Greene, M.H. Dalager, N.A., Lamberg, S.I., Arayropoulos, C.E., Fraumeni, J.F.Jr. Mycosis Fungoides: Epidemiologic Observations. **Cancer Treat Rep** 63: 597-606, 1979.

28. Fischmann, A.B., Bunn, P.A. Jr., Guccion, J.G., Matthews, M.J., Minna, J.D. Exposure to Chemicals, Physical Agents and Biologic Agents in Mycosis Fungoides and Sézary Syndrome. **Cancer Treat Rep** 63: 591-596, 1979.

29. Cohen, S., Stenn, K.S., Bravermann, I.M., Beck, G.L. Mycosis Fungoides: Clinicopathologic Relationships, Survival and Therapy in 59 Patients with Observations on Occupation as a New Prognostic Factor. **Cancer** 46: 2654-2666, 1980.

30. Poiesz, B.J., Ruscetti, F.W., Reitz, M.S., Kalyanaraman, V.S., Gallo, R.C. Isolation of a New Type C Retrovirus (HTLV) in Primary Uncultured Cells of a Patient with Sézary T-Cell Leukemia. **Nature** 294: 268-271, 1981.

31. Alibert, J.L. **Description des Maladies de la Peau Observées a l'Hôpital St. Louis et Exposition des Meilleures Méthodes Suives pour Leur Traitement.** Paris. Barois l'ainé et Fils, 1806, p.157.

32. Sézary, A., Bouvrain, Y. Erythrodermie avec Présence de cellules monstreuses dans le Derme et le Sang Circulant. **Bull Soc Fr Dermatol Syphilia** 45: 254-260, 1938.

33. Vidal, E., Brocq, L. Étude sur le Mycosis Fungoides. **France Medical** 2: 946, 957, 969, 1005, 1014, 1085, 1885.

34. Carney, D.N., Bunn, P.A. Manifestations of Cutaneous T-Cell Lymphoma. **J Dermatol Surg Oncol** 6: 369, 1980.

35. Haynes, B.F., Metzgar, R.S., Minna, J.D., Bunn, P.A. Phenotypic Characterization of Cutaneous T-Cell Lymphoma. **N Eng J Med** 304: 1319-1323, 1981.

36. Holden, C.A., Staughton, R.L.D., Campbell, W.-A., MacDonald, D.M. Differential Loss of T lymphocyte Markers in Advanced Cutaneous T-Cell Lymphoma. **J Am Acad Derm** 6: 507-513, 1982.

37. Rappaport, H., Thomas, L.B. Mycosis Fungoides: The Pathology of Extracutaneous Involvement. **Cancer** 34: 1198-1229, 1974.

38. Edelson, R.L., Berger, C.L., Raafat, J., Warburton, D. Karyotype Studies of Cutaneous T-Cell Lymphoma: Evidence for Clonal Origin. **J Invest Derm** 73: 548-556, 1979.

39. Nouvell, P.C., Finan, J.B., Vonderheid, E.C. Clonal Characteristics of Cutaneous T-Cell Lymphoma: Cytogenetic Evidence from Blood, Lymph Nodes and Skin. **J Invest Derm** 78: 69-75, 1982.

40. Shapiro, P.E., Warburton, D., Berger, C.L., Edelson, R.L. Clonal Chromosomal Abnormalities in Cutaneous T-Cell Lymphoma. **Cancer Genet Cytogenet** 28: 267-276, 1987.

41. Smith, M., Spence, M.A. Report of the Committee on the Genetic Constitution of Chromosomes 7,8 and 9. Human Gene Mapping 8. **Cytogenet Cell Genet** 40: 156-178,

1985.

42. Cox, D.R., Gedde-Dahl, T. Report of the Committee on the Genetic Constitution of Chromoses 13, 14, 15 and 16. Human Gene Mapping 8. **Cytogenet Cell Genet** 40: 206-241, 1988.

43. Blaylock, W.K., Clendenning, W.E., Carbone, P.P., Van Scott, E.J. Normal Immunologic Reactivity in Patients with Lymphoma Mycosis Fungoides. **Cancer** 19: 233-236, 1966.

44. Joyner, M.V., Cassuto, J.-P., Dujardin, P., Barety, M., Audoly, P. Cutaneous T-Cell Lymphoma in Association with A Monoclonal Gammopathy. **Arch Derm** 115: 326-328, 1979.

45. Amrein, R., Ellman, L. Sézary Syndrome with Warm and Cold Erythrocyte Autoantibodies. **Blood** 51: 1229-1230, 1978.

46. Laroche, L. Kaiserlian, D. Decreased Natural Killer Cell Activity in Cutaneous T-Cell Lymphoma. **N Eng J Med** 308: 101-102, 1983.

47. Tron, V.A., Rosenthal, D., Sauder, D.N. Epidermal IL-1 is Increased in Cutaneous T-Cell Lymphoma. **J Ivest Derm** 90: 378-381, 1988.

48. Ruscetti, F.W., Gallo, R.C. Human T-Lymphocyte Growth Factor: Regulation of Growth and Function of T-Lymphocytes. **Blood** 57: 379-394, 1981.

49. Lamberg, S.I., Green S.B., Byar, D.P. Status Report of 376 Mycosis Fungoides Patients at 4 Years: Mycosis Fungoides Cooperative Group. **Cancer Treat Rep** 63: 701-707, 1979.

50. Lutzner, M.A., Hobbs, J.W., Horvath, P. Ultrastructure of Abnormal Cells in Sézary Syndrome, Mycosis Fungoides and Parapsoriasis en Plaque. **Arch Derm** 103: 375-386, 1971.

51. Rappaport, H., Thomas, L.B. Mycosis Fungoides: The Pathology of Extracutaneous Involvement. **Cancer** 34: 1198-1229, 1974.

52. Fuks, Z.Y., Bagshqw, M.A., Farber, E.M. Prognostic Signs and the Management of Mycosis Fungoides. **Cancer** 32: 1385-1395, 1973.

53. Flaxman, B.A., Zelazney, G., Van Scott, E.J. Nonspecificity of Characteristic Cells in Mycosis

Fungoides. **Arch Derm** 104: 141-147.

54. Bendelac, A., O'Connor, N.T., Daniel, M.-T., Boitard, C., Michel, C., Laroche, L., Lesavre, P., Bach, J.F. Nonneoplastic Circulating Sézary-like Cells in Cutaneous T-Cell Lymphoma. **Cancer** 60: 980-986, 1987.

55. Guccion, J.G., Fischmann, A.B., Bunn, P.A.Jr., Schechter, G.P., Patterson, R.H., Matthews, M.J. Ultrastructural Appearance of Cutaneous T-Cell Lymphoma in Skin, Lymph Nodes and Peripheral Blood. **Cancer Treat Rep** 63: 565-570, 1979.

56. Vonderheid, E.C., Tay, E., Sobel, E.L., Schwab, E., Micaily, B., Jegasothy B.V. Clinical Implications of Immunologic Phenotyping in Cutaneous T-Cell Lymphoma. **J Am Acad Derm** 17: 40-52, 1987.

57. Ralfkiaer, E., O'Connor, N.T.J., Crick, J., Wantzin, G.L., Mason, D.Y. Genotypic Analysis of Cutaneous T-Cell Lymphomas. **J Invest Derm** 88: 762-765, 1987.

58. Lamberg, S.I., Grem, S.B., Byar, D.P., Block, J.B., Clendenning, W.E., Douglass, M.C., Epstein, E.H., Fuks, Z.Y., Golitz, L.E., Lurincz, A.L., McBurney, E.I., Michel, B., Roenigk, H.H., Van Scott, E. J., Vonderheid, E.C. Clinical Staging for Cutaneous T-Cell Lymphoma. **Ann Intern Med** 100: 187-192, 1984.

59. Schechter, G.P., Sausville, E.A., Fischmann, A.B., Soehnlen, F.,Bunn, P.A. Evaluation of Circulating Malignant Cells Provides Prognostic Information in Cutaneous T-Cell Lymphoma. **Blood** 69: 841-849, 1987.

60. Farber, E.M., Zackheim, H.S., McClintock, R.P. Treatment of Mycosis Fungoides with Various Strengths of Flucinolone Acetinide Cream. **Arch Derm** 97: 165-172, 1968.

61. Van Scott, E.J. Kalmanson, J.D. Complete Remissions of Mycosis Fungoides Lymphoma Induced by Topical Nitrogen Mustard. **Cancer** 32: 18-30, 1973.

62. Zackheim, H.S., Epstein, E.H. Jr., Grekin, D.A. Treatment of Mycosis Fungoides with Topical BCNU. **Cancer Treat Rep** 63: 623, 1979.

63. Gilchrest, B.A. Methoxsalen Therapy for Mycosis Fungoides. **Cancer Treat Rep** 63: 663-667, 1979.

64. Honigsmann, H., Brenner, W., Rauschmeir, W., Konrad, K., Wolff, K. Photochemotherapy for Cutaneous T-Cell Lymphoma. **J Am Acad Derm** 10: 238-245, 1984.

65. Parish, J.A., Fitzpatrick, T.B., Tannenbaum, L., Pathak, M.A. Photochemotherapy of Psoriasis with Oral Methoxypsoralen and Longwave Ultraviolet light. **N Eng J Med** 291: 1207-1211, 1974.

66. Song, P-S, Tapley, K.J. Jr. Photochemistry and Photobiology of Psoralens. **Photochem Photobiol** 29: 1177-1197, 1979.

67. Hoppe R.T., Cox, R.S., Fuks, Z., Price, N.M., Bagshaw, M.A., Farber, E.M. Electron-beam Therapy for Mycosis Fungoides: the Stanford University Experience. **Cancer Treat Rep** 63: 691-700, 1979.

68. Pineda, A.A., Winkleman, R.K. Leukapheresis in the Treatment of Sézary Syndrome. **J Am Acad Derm** 5: 544-549, 1981.

69. Minna, J.D., Roenigk, H.H., Glatstein, E. Report of the Committee on Therapy for Mycosis Fungoides and Sézary Syndrome. **Cancer Treat Rep** 63: 729-736, 1979.

70. Bunn, P.A. Jr., Huberman, M.S., Whang-Peng, J. Prospective Staging Evaluation of Patients with Cutaneous T-cell Lymphomas: Demonstration of a High Frequency of Extracutaneous Dissemination. **Ann Intern Med** 93: 223-230, 1980.

71. Kaye, F.J., Bunn, P.A. Jr., Steinberg, S.M., Stocker, J.L., Ihde, D.C., Fischmann, A.B., Glatstein, E.J., Schechter, G.P., Phelps, R.M., Foss, F.M., Parlette, H.L.III, Anderson, M.J., Sausville, E.A. A Randomized Trial Comparing Electron-Beam Radiation and Chemotherapy with Topical Therapy in the Initial Treatment of Mycosis Fungoides. **N Eng J Med** 321: 1784-1790, 1989.

72. Epstein, E.H. Jr, Levin, D.L., Croft, J.D.Jr., Lutzner, M.A. Mycosis Fungoides: Survival, Prognostic Features, Response to Therapy and Autopsy Findings. **Medicine** 51: 61-72, 1972.

73. Edelson, R.L. Light-Activated Drugs. **Sci Am** 259: 68-75, 1988.

74. Cohen, I.R. Regulation of Autoimmune Disease: Physiologic and Therapeutic. **Immunological Reviews** 94: 5-21, 1986.

75. Ben-Nun, A., Wekerle, H., Cohen, I.R. Vaccination against Autoimmune Encephalomyelitis with T-Lymphocyte Line Cells Reactive against Myelin Basic Protein.

Nature 292: 60-61, 1981.

76. Holoshitz, J., Naparstek, Y., Ben-Nun, A., Cohen, I.R. Lines of T-Lymphocytes Induce or Vaccinate against Autoimmune Arthritis. **Science** 219: 56-58, 1983.

77. Cohen, I.R. T-cell Vaccination against Autoimmune Disease. **Hospital Practice** Feb. 15: 57-64, 1989.

78. Khavari, P.A., Edelson, R.L., Lider, O., Gasparro, F.P., Weiner, H.L., Cohen, R.L. Specific Vaccination Against Photoinactivated Cloned T Cells. **J Clin Invest** 90: 575a, 1988.

79. Perez, M. Edelson, R.L., Laroche, L., Berger, C. Inhibition of Antiskin Allograft Immunity by Infusions with Syngeneic Photoinactivated Effector Lymphocytes. **J Invest Derm** 92: 669-676, 1989.

80. Perez, M., Gaspas, Y., O'Neil, D., Edelson, R.L., Berger, C.L. Inhibition of Murine Autoimmune Disease by Reinfusion of Syngeneic Lymphocytes Inactivated with Psoralen and UVA Light. **J Invest Derm** 86: 494a, 1986.

81. Morse, H.L.III, Davidson, W.F., Yetter, R.A., Murphy, E.D., Roth, J.B., Coffman, R.L., Abnormalities Induced by the Mutant Gene LPR: Expansion of a Unique Lymphocyte Subset. **J Immunol** 129: 2612-2615, 1982.

82. Berger, C.L. Experimental Murine and Primate Models for Dissection of the Immunosuppressive Potential of Photochemotherapy in Autoimmune Disease and Transplantation. **Yale J Biol Med** (in press).

83. Laskin, J.D., Lee E., Yurkew, E.J., Laskin, D.L., Gallo, M.A. A Possible Mechanism of Psoralen Phototoxicity not Involving Direct Interaction with DNA. **Proc Natl Acad Sci** 82: 6158-6162, 1985.

84. Abramowicz, M. (ed.) Extracorporeal Photochemotherapy for Cutaneous T-Cell Lymphoma. **The Medical Letter** 30 (776): 96, 1988.

85. Heald, P.W., Perez, M.I., Christensen, I., Dobbs, N., McKiernan, G., Edelson, R.L. Photopheresis Therapy of Cutaneous T-cell Lymphoma: The Yale-New Haven Hospital Experience. **Yale J Biol Med** (in press).

86. Edelson, R.L. Update on Photopheresis. **Yale J Biol Med** (in press).

87. Pepino, P., Berger, C.L., Fuzesi, L., Panza, A.,

Pierson, R.N., Gutierrez, C., Marboe, C.C., Smith, C.R., Reemtsma, K., Rose, E.A. Primate Cardiac Allo-and Xenotransplantation: Modulation of the Immune Response with Photochemotherapy. **Eur Surg Res** 960: 1-8, 1989.

88. Fuzesi, L., Pepino, P., Berger, C.L., Panza, A., Chiang Y-C, Marboe, C.C., Pierson, R., Smith, C.R., Reemtsma, K., Rose, E. Immunomanipulation of the Response to Cardiac Allo and Xenoreactive Leukocytes. **Transplant Proc** 21: 837-841, 1989.

89. Rook, A.H., Edelson, R.L., Heald, P.W., Nahass, G.T., Macey, W., Witner, W.K., Lazarus, G.S., Jegasothy, B.V. Treatment of Autoimmune Disease with Extracorporeal Photochemotherapy: Pemphigus Vulgaris. **Yale J Biol Med** (in press).

90. Prendeville, J., Smith, N.P., Berger, C.L., Edelson, R.L., Chu, A.L. Monoclonal Antibodies in the Tissue Diagnosis of Cutaneous T-Cell Lymphoma. **J Invest Derm** 82: 560a, 1984.

91. Meacham, R., Smith, N., Robinson, D., Chu, A.C. The Sézary Cell - An Immunological Study. **J Invest Derm** 82: 560a, 1984.

92. Ralfkiaer, E., Gatter, K.C., Wantzin, G.L., Thomsen, K., Mason, D.Y. Immunohistological Reactivity Pattern of the Anti-Cutaneous T-Cell Lymphoma Antibody BE2. **Br J Derm** 114: 677-684, 1986.

93. Medsger, T.A.Jr. Systemic Sclerosis (Scleroderma), Localized Scleroderma, Eosinophilic Fasciitis and Calcinosis in **Arthritis and Allied Conditions** 11th Ed. Edited by Daniel T. McCarty. Philadelphia, Lea & Febiger, 1989, 1118-1165.

94. Rosenbloom, J., Feldman, G., Freundlich, B., Jimenez, A. Inhibition of Excessive Scleroderma Fibroblast Collagen Production by Recombinant Gamma-Interferon. **Arth Rheum** 29: 851-856, 1986.

95. Claman, H.N. On Scleroderma: Mast Cells, Endothelial Cells, and Fibroblasts. **J Am Med Assoc** 262: 1206-1209, 1989.

96. Leder, P. The Genetics of Antibody Diversity. **Sci Am** 246: 102-115, 1982.

97. Siu, G., Clark, S.P., Yoshikai, Y., Malissen, M., Yanagi, Y., Strauss, E., Mak, T.W., Hood, L. The Human T Cell Antigen Receptor is Encoded by Variable ,

Diversity, and Joining Gene Segments that Rearrange to Generate a Complete V Gene. **Cell** 37: 393-401, 1984.

98. Cleary, M., Chao, J., Wornke, R., Sklar, J. Immunoglobulin Gene Rearrangement as a Diagnostic Criterion of B-cell Lymphoma. **Proc Natl Acad Sci** 81: 593-597, 1984.

99. Sklar, J., Cleary, M.L., Thielemans, K., Gralow, J., Warnke, R., Levy, R. Biclonal B-cell Lymphoma. **N Eng J Med** 311: 20-27, 1984.

100. Saito, H., Kranz, D.M., Takagaki, Y., Hayday, A.C., Eisen, H.N., Tonegawa, S. Complete Primary Structure of a Heterodimeric T-Cell Receptor Deduced from cDNA Sequences. **Nature** 309: 757-762, 1984.

101. Kim, S., Davis, M., Sinn, E., Patten, P., Hood, L. Antibody Diversity: Somatic Hypermutation of Rearranged V_H Genes. **Cell** 27: 573-581, 1981.

102. Southern, E.M. Detection of Specific Sequences among DNA Fragments Separated by Gel Electrophoresis. **J Mol Biol** 98: 503-517, 1975.

103. Minden, M.D., Mak, T.W. The Structure of the T Cell Antigen Receptor Genes in Normal and Malignant T Cells. **Blood** 68: 327-336, 1986.

104. Berliner, N., Duby, A.D., Linch, D.C., Murre, C., Quertermous, T., Knott, L.J., Azin, T., Newland, A.C., Lewis, D.L., Galvin, M.C., Seidman, J.G.. T Cell Receptor Gene Rearrangements Define a Monoclonal T Cell Population in Patients with T Cell Lymphocytosis and Cytopenia. **Blood** 67: 914-918, 1986.

105. Tawa, A., Hozumi, N., Minden, M., Mak, T.W., Gelfand, E.W., Rearrangement of the T-Cell Receptor ß Chain Gene in Non-T, Non-B Acute Lymphoblastic Leukemia. **N Eng J Med** 313: 1033-1037, 1986.

106. Smith, L.J., Curtis, J.E., Messner, H., Senn, J.S., Furthmayr, H., McCulloch, E.A. Lineage Infidelity in Acute Leukemia. **Blood** 61: 1138-1145, 1982.

107. Greisser, H., Tkachuk, D., Reis, M.D., Mak, T.W. Gene Rearrangements and Translocations in Lymphoproliferative Diseases. **Blood** 73: 1402-1415, 1989.

108. Cheng, G.Y., Minden, M.D., Toyonaga, B., Mak, T.W., McCullough, E. T-Cell Receptor and Immunoglobulin Gene

Rearrangements in Acute Myeloblastic Leukemia. **J Exp Med** 163: 414-424, 1986

109. Arnold, A., Cossman, J., Bakhshi, A., Jaffe, E.S., Waldmann, T.A., Korsmeyer, J.J. Immunoglobulin Gene Rearrangements as Unique Clonal Markers in Human Lymphoid Neoplasms. **N Eng J Med** 309: 1593-1599, 1983.

110. Berger, C.L., Eisenberg, A., Stoper, L., Chow, J., Simone, J., Gaspas, Y., Cacciapaglia, B., Bennett, L., Edelson, R., Warburton, D., Benn, P. Dual Genotype in Cutaneous T Cell Lymphoma: Immunoglobulin Gene Rearrangement in Clonal T Cell Malignancy. **J Invest Derm** 90: 73-77, 1988.

111. Rigby, P., Dieckmann, M., Rhodes, C., Berg, P. Labeling Deoxyribonucleic Acid to High Specific Activity in Vitro by Nick Translation with DNA Polymerase I. **J Mol Biol** 113: 237-251, 1977.

112. Maniatis, T., Fritsch, E., Sambrook, J. **Molecular Cloning: A Laboratory Manual.** Cold Spring Harbor Laboratory, Cold Spring Harbor NY, 1982.

113. Hafler, D.A., Fox, D.A., Manning, M.E., Schlossman, S.F., Reinherz, E.L., Weiner, H.L. In Vivo Activated T Lymphocytes in the Peripheral Blood and Cerebrospinal Fluid of Patients with Multiple Sclerosis. **N Eng J Med** 312: 1405-1410, 1985.

114. Hemler, M.E., Glass, D., Coblyn, J.S., Jacobson, J.G. Very Late Activation Antigens on Rheumatoid Synovial Fluid T Lymphocytes. **J Clin Invest** 78: 696-702, 1986.

115. Stamenkovic, I., Stegagno, M., Wright, K.A., Krane, S.M., Amento, E.P., Colvin, R.B., Duquesnoy, R.J., Kurnick, J.T. Clonal Dominance among T-Lymphocyte Infiltrates in Arthritis. **Proc Natl Acad Sci** 85: 1179-1183.

116. Keystone, E.C., Minden, M., Klock, R., Poplonski, L., Zalcberg, J., Takadera, T., Mak, T.W. Structure of T-cell Antigen Receptor ß Chain in Synovial Fluid Cells from Patients with Rheumatoid Arthritis. **Arth Rheum** 31: 1555-1557, 1988.

117. Tesch, H., Hohlfeld, R., Toyka, K.V. Analysis of Immunoglobulin and T Cell Receptor Gene Rearrangements in the Thymus of Myasthenia Gravis Patients. **J Neuroimmunol** 21: 169-176, 1989.